Studies into factors affecting fruit production in young apple trees

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STUDIES INTO FACTORS AFFECTING FRUIT PRODUCTION IN YOUNG APPLE TREES.

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SUMMARY

The causes of poor fruit production on young apple trees, and possible methods of overcoming them were investigated with particular reference to Cox's Orange Pippin grown in non-intensive planting systems.

Assessment of the components of cropping within trees of various ages indicated poor fruit set, rather than lack of flowers, to be the main limitation on fruit production.

Female fertility and the length of the effective pollination period were both very much reduced in flowers on young trees, 1-year-old wood and vertical branches compared to those on older trees, older wood and horizontal branches respectively. The advantage conferred to fowers by a horizontal branch orientation appeared to be effected during the period of flowering/fruit set rather that prior to this.

Detailed investigations to determine which component(s) of fruit set were limiting in flowers where female fertilty and effective pollination period were low showed that the manner and rate in which stigmatic surfaces degenerated with time, and the number and rate of pollen tubes growing through the style was similar within flowers of very different setting abilities. Both of these declined as time beyond anthesis increased but they did not vary consistently between flowers of different setting abilities. However, at all times of assessment throughout the flowering period, flowers from young trees, 1-year-old wood and vertical branches had fewer healthy egg-sacs than did those from older trees, older wood and horizontal branches respectively. This was due to a higher proportion being either immature or overmature. Following pollination at increasing lengths of time after 'late balloon' there were very few healthy developing embryos within the fruitlets collected from these former situations.

No consistent differences in cluster development, mineral or chlorophyll content were found between flowers of different setting abilities.

Neither the time of floral initiation nor the rate of floral differentiation were found to differ between buds on 2-year-old wood on any age of tree; those on current years wood initiated later. Time of floral initiation did not appear related to the time of shoot growth cessation.

Both paclobutrazol and daminozide applications increased the numbers of fowers produced and fruitlets set but also severely reduced vegetative growth. Training branches to a horizontal position also increased the numbers of flowers and fruits produced, but without such severe growth reduction. Shoot tipping had variable results but did not significantly increase cropping.

CONTENTS

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Chapter 1. General introduction.

1.1 Economics of orchard establishment.

Historically, fruit orchards have taken many different forms ranging from elaborately trained and pruned dwarf trees in monastery and palace gardens to the large and spreading trees under which cattle grazed. The natural lifespan of these trees was in the order of 100-150 years, the first part being devoted to vegetative growth rather than fruit production. During the last half century however, commercial fruit production has greatly intensified.

Originally apple and pear trees were grown on their own roots and grew naturally to a large size but since the early part of this century those in commercial plantings have been grown on rootstocks. Propagation of these trees is vegetative, buds of cultivar trees (scions) being grafted onto the clonal rootstock. Such rootstocks influence the size to which a tree will grow, and their introduction brought the option to have smaller mature apple trees. There are now several commonly used rootstocks, each of which confer a different vigour and size to the scion. These range from M27 (giving very small, compact trees) to M2 (large and vigorous), with M9 (dwarfing) and MM106 (slightly larger) being intermediate and the most popular rootstocks in Britain and Northern Europe. M26, less vigorous than MM106, is also widely used in Europe and the U.S.A. As the size of individual trees became controlled and gradually reduced within commercial orchards, planting density tended to increase.

The most widespread effect of this trend towards reduction in tree size has been the 'spindle bush' - a small tree about 2 metres in height and 1.5 metres in width, cropping on horizontal fruiting laterals. It was developed in Germany during the 1930's, and extensively planted throughout Northern Europe - especially in Holland - at spacings of 4m x 2m. Since the 1960's, these trees have been progressively replaced by even more compact ones - slender spindles - initially at spacings ranging between 3m x 1m to 4m x 2m but now, with the introduction of multi-row bed systems, often much closer.

One advantage of the higher density plantings is that only limited vegetative growth is needed to fill orchard space and a greater proportion of resources can therefore be directed into fruit production. High density orchards may crop within 1-2 years from planting, the greatest precocities reported to date being achieved within experimental 'meadow-orchards' planted at 70,000 trees/ha (Luckwill and Child 1973), and with trees on the very dwarfing rootstock M27, planted at 13,720 trees/ha (Preston 1978); in both cases fruit yields of 50t/ha were obtained in the second year after planting. The general implications of high density plantings have been discussed by Goedegebure (1980) who showed that yield/ha could be continuously increased as plant density increased from 1,000 - 3,000 trees/ha.

Although the yield of individual trees is low when they initially start the reproductive phase, in high density plantings the cumulative yield per hectare can be high within a few years from

planting (Parry 1981). As high and low density orchards mature, the initially much higher yields per hectare obtained in the former are usually retained as such even though individual trees eventually yield more in the latter (Westwood *et al.* 1976, Goedegebure 1980, Parry 1981). Overall these orchards appear very attractive in that they crop early, thus minimising unproductive time, and they then maintain high levels of production.

However, there are disadvantages to this system; the cost of establishing such orchards being high compared to the more conventional less dense ones. Within Britain, commonly used planting distances are around 5m x 3m or 4.5m x 2m; spacings which have 667 and 1111 trees/hectare respectively. In comparison, the 'North Holland' system - very popular in Europe - involves planting trees in 3 close rows in between adjacent alleys and requires around 3,000 trees/hectare.

Given that the cost of a single tree (in 1982) was around £1.80, and that each required a stake (larger and more expensive for the smaller trees within the more dense plantings), the cost of materials alone for planting an orchard at the three densities detailed above would have been £1,921, £3,567 or £9,681 per hectare respectively (Steer 1984). Thus until the cost of individual trees is reduced, or government policy regarding interest rates and finance in general for replanting is changed, low density plantings will appear financially attractive.

In addition, planting at high density is sometimes not practicable in terms of management. By their very nature, high density orchards have small allocated spaces between trees and because of this, growth must be carefully controlled. In certain British fruit growing areas (e.g. East Anglia), the heavy soil type encourages vigorous vegetative growth such that trees cannot be retained within their alloted space and orchards become uncontrolled.

Thus at present, in order to avoid both the high establishment costs and potential management problems associated with high density plantings, British growers often plant at around 700 trees/ha using large well feathered Cox trees on the semi-vigorous MM106 rootstock to encourage early filling of space (Jackson *et al.* 1986, A.D.A.S pers. comm.).

However, this does leave growers with a problem. The lower density plantings, although having proportionally lower establishment costs, are slower to crop and produce returns (Parry 1981). Ideally the vegetative growth needed to fill an orchard with cropping wood would occur rapidly alongside fruit production but unfortunately, for reasons discussed in the following section, this is unlikely to happen and orchards may remain unproductive, or with very low production, for the first few years after planting.

1.2 Flowering and fruit production in young trees.

One main problem of cropping within a young orchard is that flowers can be slow to be produced (Vienbrants 1972). Without flowers, no fruit can be produced and the orchard remains unfruitful.

Because fruit trees are vegetatively propagated from trees which have already passed through their juvenile phase (during which they are physiologically incapable of flowering) this lack of flower production is not caused by juvenility, but rather by lack of precocity.

Although the length of the non-fruiting stage in clonally propagated material is related to the length of the juvenile phase in young seedlings of the same species, it is usually several years shorter (Tydeman 1967, Tydeman and DeVries 1970).

To distinguish between juvenility and precocity, Wareing (1959) described the phase change from juvenile to adult as 'maturation' whereas the loss of vigour and subsequent flowering associated with the development of the tree was termed 'ageing'. One obvious difference between the two phenomena is that 'ageing' and the resultant precocity are highly susceptible to environmental influences whereas the time taken to achieve 'maturation' is not (Visser 1967).

Thus, in theory, young fruit trees should immediately be capable of producing flowers and fruit, but in practice rarely do so without drastic manipulation. Young trees can be induced to crop but the most successful and reliable methods of achieving this are those which also severely restrict vegetative growth. Obviously such methods are unsuitable in low-density orchards where growth is desired; but the findings do suggest one area where the problem of poor precocity in these orchards might lie.

At planting, young trees have relatively few growing points, each of which is inclined to grow strongly. As shoot-tips grow, auxin and gibberellin produced by the young expanding leaves (Phillips 1975, Tromp and Wertheim 1980) is transported down to the roots where they stimulate growth and the production of hormones and metabolites. These are then translocated back up into the shoot-tips for use in continued growth, more auxin and gibberellin are subsequently produced and the cycle continues. Because young trees have so few shoot tips, each can attract a large proportion of available metabolite, and consequently, each can grow strongly. Because each tip is growing so vigorously, the strong apical dominance exerted by each inhibits any subtending lateral buds from growing out (Nasr and Wareing 1961); consequently young trees often have extremely vigorous growth of few, unbranched shoots.

It is widely recognised that vegetative growth is antagonistic to flower initiation both for fruit trees (Davis 1957) and plants in general (Lang 1961). It is also considered to be the main cause of poor flowering and cropping within young orchards where initial vegetative growth is needed to fill allocated space (Forshey 1978).

Unlike the situation with photoperiodic or cold requiring herbaceous plants, flower induction in fruit trees is not triggered by an externally mediated all-or-none process. Rather the process is complex and undoubtedly involves the interaction and balance of several endogenous growth regulators.

In Cox and other spur bearing cultivars, the majority of flower buds are formed within buds borne on 2-year-old or older wood, each of which is capable of developing into either a vege-

tative shoot or a flower cluster. At the start of the season a bud may comprise one immature scale plus five or six leaf primordia, but as the season progresses it increases in complexity by adding more primordia at the apex whilst older primordia become transformed into further bud scales or transition leaves (Abbott 1977). Although it has been shown that the bud must produce at least 20 nodes before it reaches sufficient complexity to form either a leafy shoot or flower initials (Luckwill 1974, Abbott 1977), it is not only the absolute number of these which is important but also the time interval between two successive primordia being formed, a period known as the plastochron (Fulford 1966). A strongly growing bud apex may produce new primordia at a rapid rate, reach 20 nodes very quickly, and grow out as a leafy shoot in the current year (Abbott 1977). A weakly growing point may add primordia so slowly that by the time dormancy sets in it has not reached sufficient complexity for floral induction and would thus be retained as a vegetative bud. In apple, a plastochron of more than eight days is associated with buds which remain vegetative whereas one of around five days will result in floral initiation occurring in early August (Abbott cited in Luckwill 1974).

Thus the state of activity within the bud determines whether or not flowers will be formed and it is this activity which is supressed by vigorous shoot growth.

Because young expanding leaves produce large amounts of gibberellin (Kato and Ito 1962, Tromp and Wertheim 1980), and because applications of this hormone usually decrease flower production (Luckwill and Silva 1979, Tromp 1982), it is believed that the inhibition of flowering associated with vigorous shoot growth may be effected through gibberellin (Tromp and Wertheim 1980). The theory suggested is that while levels of gibberellin within the shoot remain high, activity within subtending buds is inhibited such that they have a long plastochron and consequently do not develop enough complexity to develop either as a shoot or as floral primordia. Only when shoot growth slows down (perhaps as a result of cooling air temperatures) and gibberellin levels fall, are the buds released from this inhibition. If this happens while the soil is still warm and the roots are still maintaining growth and metabolite production, then with no active shoot tips to attract them, metabolites will instead be directed towards the lateral buds with the best vascular connections (Abbott 1984). Here they can stimulate activity and shorten the plastochron. Because little gibberellin is present, the bud does not elongate into a shoot once it reaches the 20 node stage, but rather it initiates flower primordia. However, if shoot growth continues late into the season, by the time gibberellin levels have fallen sufficiently to release the buds from inhibition, roots may also have stopped growing and cytokinin levels may have fallen below the level required to stimulate activity within the buds. In this situation buds would enter dormancy at their current developmental stage, no flowers or shoots being produced from them that year.

Because of the small number of shoots present on young trees and the plentiful metabolite supply to each, growth does usually continue later into the season than occurs on older trees.

As such there is a far greater chance of buds not being released from inhibition during the time that cytokinin is available, or, if they are, this may happen so close to winter that even if floral initiation occurs, only limited pre-dormancy development will be possible. Although flowers will be present the following year, they will be physiologically 'young', a situation which Abbott (1970) showed to give rise to 'weak' blossom which set poorly. Thus fruit would still not be produced and the situation of excessive vigour and low productivity would continue. Thus it is not only the presence of flowers *per se* which ensures fruit production, the flowers produced must be of sufficient 'quality' or 'strength' to be capable of setting fruit. Flowers with unreceptive stigmas or malformed/degenerate ovules will effectively be sterile, incapable of developing into fruits. Similarly, an inadequate supply of nutrients (either from cluster leaves or from within the main tree) will be insufficient to maintain the growth and development of flowers and/or developing fruits. Consequently flowers may abscise before fertilisation, or fruitlets abort and then abscise.

It can therefore be seen that only when excess vigour is reduced will both lateral shoots and good 'quality' flower clusters be produced, and the tree come into cropping. Ironically, once fruitlets are present on a tree the problem of excessive growth and low flower production is likely to be greatly reduced. Developing fruitlets compete very strongly against the shoot tips for available metabolites, (Maggs 1963) thereby making less available for each one. This limits the rate of growth, reduces the gibberellin levels and thus releases lateral buds from inhibition early in the season. If this happens early enough with a plentiful supply of cytokinin still available, some of the buds will quickly grow out as shoots, thus creating yet more 'sinks' and further drawing metabolites away from the original vigorous shoots. By this sequence of events the tree settles down into a steady cropping state where early in the season shoot growth proceeds alongside flowering and fruit set. Then, as fruitlets are formed and generate their own supply of hormones, nutrients needed to support fruit growth and development are attracted towards them, making fewer available for each shoot tip. Consequently, growth slows down, releasing buds from inhibition and allowing them to initiate floral primordia relatively early in the season while there is still time for them to develop well. Good 'quality' flowers will then be formed the next year; these will be able to set well and the balanced cycle of growth and fruit production will be maintained.

Thus, mature trees are capable of maintaining a good balance between growth and fruiting, but in young trees the problem of how to encourage the vegetative growth needed to fill orchard space, whilst concurrently allowing lateral buds to be released from inhibition such that they can initiate flowers and start the tree cropping, remains.

Given that there are many, varied methods of increasing the flower and fruit production of mature trees, it is possible that some of these could also be used beneficially on young trees. However, because there is only limited understanding of all the factors which affect the vege-

tative and reproductive growth of young trees, several attempts to increase precocity have met with unsatisfactory results. In some cases, treatments were apparently ineffective (Greene and Lord 1978); in others, although precocious fruiting was achieved, vegetative growth was much reduced (Luckwill and Child 1973) and early filling of orchard space would have been inhibited. Although no proven, reliable method of increasing precocity whilst maintaining growth exists at present, it is not necessarily an impossiblity, but further investigations need to be conducted in order to determine the potential for this.

1.3 Methods of improving productivity of mature trees.

Techniques presently available for improving orchard productivity can be classified as genetical, cultural or chemical (Luckwill 1970).

Of the genetic methods, the use of clonally dwarfing rootstocks has proved to be the cheapest and most effective way of controlling tree vigour and increasing the flower bud production and fruit set of young trees (Tubbs 1967). But although rootstocks are almost invariably used within commercial fruit production, and they have improved precocity they have not completely solved the problem of fruitless young orchards.

Within the second category - cultural methods - techniques reported to increase flower and/or fruit production are:

a) Scoring -- cutting through the bark of individual limbs or whole trunks (Southwick *et al* 1967, Veinbrants 1972).

b) Girdling/ringing -- where small strips of bark are removed from around a branch or trunk (Ferree and Palmer 1982).

c) Shoot-tipping -- removal of shoot tips at one or more times during the growing season (Quinlan and Preston 1971, Grauslund 1978).

d) Branch bending -- where naturally upright branches are physically trained to a horizontal or downswept position (Tromp 1968, 1972, 1987).

Branch bending in particular has gained in popularity in recent years and indeed the 'spindle-bush' system, so popular throughout Northern Europe, involves the systematic tying down of upright branches (Goldschmidt and Delap 1950). References to the effectiveness of this technique in increasing fruiting have appeared throughout the literature from Langley (1729) and Knight (1803) to numerous more recent works (e.g. Elfving and Forshey 1976, Tromp 1987).

Experimental investigations into this phenomenom have often confirmed the earlier observations (Myers and Ferree 1983, Tromp 1987) but some workers have found that the orientation of an apple tree branch bears little relation to its subsequent flowering and fruiting (Dermine and Monin 1960, Jonkers 1962, Mika 1969). However, there is good evidence that in controlled conditions gravity does affect vegetative growth and that a horizontal orientation can stimulate flowering in apple and other fruit crops (Wareing and Nasr 1958).

Within the third category of techniques, that of chemical methods, there are many quite different synthetic organic compounds available. The first to be used in an attempt to control tree growth was 2,2-dimethylsuccinamic acid (Batjer *et al.* 1964). Since then it has been extensively tested and widely used to restrict growth and enhance fruiting. Under its common names Alar, daminozide or B9, it is reported to increase both flower production (Williams 1972, Schumacher *et al.* 1978) and fruit set (Forshey 1970, Luckwill and Child 1973). Usually there are concurrent decreases in shoot growth.

Other chemicals reported to increase fruiting are paclobutrazol (Stinchcombe *et al.* 1984, Greene 1986), ethephon (Williams 1972, Greene and Lord 1978) and polyamines (Costa and Bagni 1983). Again, although there are some reports of increased fruiting with no associated decrease in shoot growth (Greene and Lord 1978), increased fruiting is usually accompanied by decreased growth, as with daminozide.

1.4 Aims of this study.

Within Britain there is a need to improve the cropping of young orchard trees, whilst simultaneously maintaining continued shoot growth. This project therefore set out to investigate the factors which limit fruit production on young, actively growing trees particularly in Cox's Orange Pippin, (Cox) and to explore the potential for increasing their precocity.

To this end, a broad investigation was conducted which examined various aspects of growth, flowering and fruit set, and the manner in which these were affected by chemical and cultural treatments. Firstly the fruit production characteristics of Cox trees of various ages (from newly planted to 12-year-olds) were monitored in order to identify the points at which the production of fruit is limited in young trees (Chapter 2), and concurrent with this, several commonly used methods of encouraging the fruiting and/or growth of older trees were investigated as to their potential within young orchards (Chapter 3).

Secondly, detailed studies were made of factors affecting fruit production both within the various ages of tree but also within different situations within individual trees (age of wood, orientation of branch). These additional situations were chosen because within each, the fruit setting ability of flowers can vary markedly. Although flowers are usually produced on all ages of wood within mature Cox trees, those on 1-year-old wood rarely set fruit whereas those on older wood can do so readily. Similarly, in line with reports suggesting that horizontal branches are more productive than vertical branches (Preston 1974), an initial experiment (described in Chapter 5, section 5.3.1) had shown that flowers on horizontal branches consistently set fruit more successfully than did flowers on vertical branches. These circumstances where differences in setting abilities of particular flowers could be predicted with reasonable confidence provided an opportunity for identifying the factors which might be responsible for the differential set itself.

Consequently, the development and nutritional content of clusters from all situations was assessed between 'bud burst' and 'anthesis' (Chapter 4). Additionally, assessment was made of the fruit setting ability of flowers borne in each of these, and additional, situations whereby the female fertility and the length of time during which pollination would result in fruitlet formation were assessed (Chapter 5). Following this, the individual components which contribute to fruit set (stigmatic condition, pollen tube growth, ovular condition and embryo development) were closely examined to identify which ones were limiting (Chapter 6).

Finally, shoot growth, floral initiation and floral development were assessed within trees of different ages, branches of different orientations and trees treated with plant growth regulators (Chapter 7).

Chapter 2. Assessment of the components of fruit production on various ages of tree.

2.1 Introduction.

2.1.1 Fruit production problems of young orchards.

Fruit orchards are long term investments. The cost of their establishment is high and it is important that fruit, and the economic returns that result from their sale are achieved as early as possible. High density orchards may crop within 1-2 years (Luckwill and Child 1973, Preston 1978) but planting at such high density is sometimes not practicable in terms of establishment costs and/or management.

However, lower density plantings, though having proportionally lower planting costs and perhaps easier management, are slower to crop and produce returns (Parry 1981).

At present there is little detailed knowledge regarding the poor fruit production of young trees but rapidly growing trees are often reported to produce few flowers and/or fruit (Gardner *et al.* 1952, Luckwill 1970, Elfving 1976). Vigorous extension growth is reported to be antagonistic to fruitfulness (Lang 1961, Forshey 1978) and any cultural methods which encourage vigorous canopy extension can delay the onset of productivity (Batjer *et al.* 1963, Elfving and Forshey 1976). Although flower bud production on young trees can be poor, and can therefore be a large contributor to cropping problems, it should not be thought of in isolation. Flower production is only one component of fruit production and indeed, in a study of the factors affecting poor cropping in 'Delicious', Dennis (1981) found flower number to be the component of fruit production least correlated with yield. The other components are initial fruit set, and then fruitlet retention and development through to maturity. All three components must be operating well in order to achieve fruit production, but it is quite possilble that although one or two may be perfectly adequate, poor performance of the other(s) may maintain low fruit production. Consequently enhancement of fruit bud production on young apple trees has not always resulted in increased numbers of fruit (Greene and Lord 1978). From the reports by Fletcher (1900) and Wallis (1911) through to more recent literature, (Gardner *et al.* 1952, Forshey 1978) there are references to the fact that although young trees may blossom well, they fail to produce any fruit.

Similarly it has been stated (Dennis 1981) that in mature trees it is the proportion of flower buds which successfully set fruit that is the most important factor governing yield. Whether this is true within young trees, and how it might change as a tree ages is unknown.

Consequently, to determine exactly which stages limit fruit production in young trees and how these are affected by tree age, the components of fruit production in various ages of trees were studied over two years. During this investigation, assessment was made of flower

bud number, the proportion of these which initially set and the numbers of fruitlets present at initial and final set on Cox's Orange Pippin (Cox) trees aged between 2- and 12-years-old.

2.2 Materials and methods.

2.2.1 Orchard material.

The experimental trees observed are shown in Table 2.2.1. and were maintained by a standard crop protection/fertilizer programme. All trees were grown in a 2 metre wide herbicide-treated strip with mown grass alleys and were pruned to a free spindle form. Vigorous upright branches were usually tied or tucked to a more horizontal position in the second or third year after planting.

2.2.2 Factors affecting fruit production within various ages of tree.

In order to assess how the components of fruit production varied according to tree age, the following measurements were made on the trees described in section 2.2.1:

(i) Number of fruit buds per tree
(ii) Number of fruitlets set 21 days after full bloom (initial set)
(iii) Number of fruitlets remaining 50 days after full bloom (final set)
(iv) Seed number in harvested fruit
(v) Total and mean shoot extension growth
(vi) Tree crown volume at start and end of 1986 season
(vi) Girth 15 cm. above the trunk union.

2.3 Results

2.3.1 Factors affecting fruit production within various ages of tree.

When assessing flower and fruit production of apple trees it is common to account for and effectively remove some of the size variation between individual trees by using girth, or trunk cross sectional area as a covariate in the statistical analysis (Williams *et al.* 1980, Wagenmakers 1988). Although Moore (1978) found a strong relationship to exist between trunk girth and the dry weight of the vegetative tree parts, he found this to hold true only after trees had been growing for more than three years. Similarly, the present experiment casts further doubts on its use when comparing very young trees with semi-mature ones. When data from the various tree ages was analysed with trunk girth included as a covariate, the adjusted means for initial and final fruit set in the youngest trees were negative very large and as such highly unreliable. Use of a covariate in statistical analysis is based on the assumption that it will vary linearly with the parameter in question. Although within trees of roughly similar physiological condition the relationship between girth and the vegetative weight may hold, there are several reasons to be wary of its use when comparing trees of different ages and stages of maturity. Young trees rapidly increase the amount of wood within their canopy, and simulta-

Table 2.2.1. Cox/MM106 orchards used in experiments described in 2.3.1. and 5.3.2.

year planted	planting distance (m.)	alley width (m.)	pollinator cultivars	number of trees used	
				1985	1986
1984	5 · 0	4 · 0	Spartan, Discovery	--	24
1983	5 · 0	4 · 0	Spartan, Discovery	24	24
1982	3 · 0	4 · 5	Spartan	16	--
1981	4 · 5	5 · 25	Discovery	12	12
1979	4 · 5	5 · 25	Katya	12	12
1976	3 · 7	4 · 6	Millers Seedling	12	--

neously increase girth. During the first few orchard years the only removal of wood from them is through very light pruning. As trees mature, pruning becomes more intensive and eventually a fairly stable amount of cropping wood is maintained. However, even when this stage has been reached and the cropping canopy is not being expanded, girth continues to increase. Thus trees which have completed the initial vigorous growth and achieved cropping stablility probably have a very different relationship to their girth than do young, rapidly growing trees. Experimental work has shown that where shoot growth has been affected by treatments this can be inversely related to girth increment (Greene and Lord 1983). This might suggest that as young trees put a large proportion of their resources into extension growth they produce less girth increment than do trees with a lower extension growth rate where a greater proportion of metabolites are available for increase in girth diameter.

Bearing all this in mind, although originally intending to use girth measurements to correct for tree size this has not been done, and as a consequence, data presented here are the mean values on a direct 'per tree' basis. Although this perhaps makes comparison of actual numbers of flowers produced and fruits set fairly academic, comparisons of the number of fruits set per 100 flower clusters and the percentage of fruits retained between initial and final set remain valid and useful. In a further attempt to take tree size into account, tree crown volume was measured at the start and finish of the 1986 growth season, and the number of flowers and fruits produced expressed relative to the final measurement. However, the usefulness of this tree crown volume is also disputable in that it is only an outer volume which takes no account of the density of wood within.

In 1985, with trees aged between 2- and 6-years-old, as the age of tree increased the number of fruit buds produced per tree also increased (Table 2.3.1.1). 6-year-old trees produced approximately 16 times as many fruit buds as did 2-year-old trees (650 and 53 buds per tree respectively), and 3 and 2.5 times as many as the 3- and 4-year-old trees respectively. 12-year-old trees were intermediate between the 4- and 6-year-old trees and produced an average of 482 buds.

In 1986, the 5- and 7-year-old trees produced similar numbers of fruit buds (459 and 511 respectively), approximately 15 times as many as were formed on the 2-year-old trees (46 buds per tree) (Table 2.3.1.2).

The number of fruits initially set also followed this pattern in both years, more fruit being set as the age of tree increased. The one exception to this was in 1985 where numbers of fruit initially set on the 12-year-old trees were intermediate between the number set on the 4- and 6-year-old trees.

This rise in numbers of fruit set with increasing tree age was not merely a reflection of the varying numbers of fruit bud initially produced, In both years, with the exception of the 12-

Table 2.3.1.1. Flower and fruit production of Cox/MM106 trees of various ages (1985). Within any row, values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

	age of tree (years)				
	2	3	4	6	12
fruit bud number (S.E.M.)	53 · 4a (4 · 9)	174 · 1b (15 · 0)	252 · 8c (16 · 2)	650 · 1e (45 · 7)	482 · 4d (27 · 7)
initial set (S.E.M.)	8 · 1a (2 · 2)	162 · 0b (24 · 1)	298 · 5c (42 · 2)	853 · 5e (87 · 5)	524 · 2d (58 · 0)
final set (S.E.M.)	1 · 8a (0 · 5)	- -	171 · 3b (26 · 0)	471 · 1d (34 · 4)	255 · 8c (30 · 8)
intitial set/100 clusters (S.E.M.)	14 · 2a (3 · 9)	103 · 7b (15 · 8)	116 · 2bc (13 · 4)	139 · 3c (18 · 3)	108 · 3b (9 · 6)
final set/100 clusters (S.E.M.)	3 · 5a (1 · 0)	- -	59 · 1b (3 · 9)	78 · 6c (7 · 8)	53 · 0b (5 · 3)
seed number (S.E.M.)	5 · 03a (0 · 22)	- -	5 · 8b (0 · 22)	6 · 11b (0 · 19)	6 · 84c (0 · 23)
% of initial set retained until final set (S.E.M.)	30 · 1a (8 · 1)	- -	59 · 4b (1 · 0)	59 · 9b (4 · 3)	49 · 2b (3 · 5)
total shoot growth (m.) (S.E.M.)	13 · 96a (1 · 46)	43 · 06b (5 · 55)	67 · 32c (9 · 85)	95 · 74d (8 · 67)	53 · 28b (6 · 83)
mean shoot length (cm.) (S.E.M.)	33 · 7a (1 · 4)	28 · 2b (1 · 6)	32 · 1a (1 · 6)	30 · 8a (1 · 7)	31 · 6a (1 · 4)
number of shoots per tree (S.E.M.)	41 · 2a (2 · 6)	152 · 1b (18 · 7)	209 · 3c (24 · 2)	311 · 3c (32 · 1)	171 · 4b (20 · 3)

Table 2.3.1.2. Flower and fruit production of Cox/MM106 trees of various ages (1986). Within any row, values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

	age of tree (years)			
	2	3	5	7
fruit bud number	46 · 1a	97 · 1b	459 · 1c	511 · 5d
(S.E.M.)	(6 · 0)	(11 · 7)	(51 · 7)	(73 · 2)
initial set	10 · 0a	16 · 8a	390 · 1b	768 · 0c
(S.E.M.)	(2 · 3)	(3 · 2)	(64 · 4)	(104 · 9)
final set	8 · 4a	13 · 9a	224 · 7b	519 · 2c
(S.E.M.)	(2 · 3)	(2 · 5)	(20 · 7)	(63 · 5)
intitial set/100	19 · 9a	31 · 4a	88 · 8b	174 · 5c
clusters (S.E.M.)	(3 · 8)	(7 · 4)	(10 · 3)	(25 · 5)
final set/100	18 · 9a	23 · 4a	56 · 2b	120 · 6c
clusters (S.E.M.)	(4 · 9)	(4 · 8)	(8 · 2)	(18 · 7)
seed number	5 · 33a	5 · 51a	5 · 56a	6 · 66b
(S.E.M.)	(0 · 24)	(0 · 30)	(0 · 32)	(0 · 32)
% of initial set retained	91 · 1b	84 · 7b	65 · 7a	71 · 6a
until final set (S.E.M.)	(3 · 5)	(3 · 8)	(5 · 8)	(4 · 8)
total shoot growth (m.)	26 · 5a	27 · 5a	71 · 8b	86 · 6c
(S.E.M.)	(1 · 2)	(3 · 5)	(8 · 4)	(8 · 6)
mean shoot length (cm.)	41 · 4a	36 · 9b	31 · 6c	31 · 3c
(S.E.M.)	(1 · 2)	(2 · 4)	(1 · 8)	(1 · 6)
number of shoots per tree	65 · 1a	74 · 2a	232 · 6b	281 · 3b
(S.E.M.)	(6 · 2)	(8 · 7)	(32 · 1)	(27 · 3)

year-old trees, the number of fruitlets set per 100 clusters also increased positively with tree age.

In 1985, the number of fruits set per 100 clusters (percentage fruit set), was greater than 100 on the 3-, 4-, 6- and 12-year-old trees, indicating that more than one of the several flowers within each fruit bud had set fruit. On the 2-year-old trees however, only 14.2 fruits were set per 100 clusters, approximately one tenth of the number set by the 6-year-old trees. There was a steady rise in percentage set as tree age increased between 2- and 6-years-old. 6-year-old trees had a significantly higher percentage fruit set than did any other tree age; that on the 2-year-olds significantly lower.

In 1986, percentage fruit set increased steadily and significantly with each rise in tree age. Approximately 20 fruit per 100 clusters were produced on the 2-year-old trees, whereas 159 fruits/100 clusters were formed on the 7-year-old trees. It was notable that the 3-year-old trees in 1985 had a much higher percentage set (104%) than did the 3-year-olds in 1986 (31%).

Although (omitting the 12-year-old trees in 1985) the numbers of fruit buds formed and fruitlets set consistently increased with tree age in both years, the magnitude of this rise varied between years. In 1985, of all tree ages examined 6-year-old trees had set the highest numbers of fruitlets, an average of 853 fruitlets being set per tree; this was an approximately hundredfold increase over the number set on the 2-year-old trees (8.1 per tree). In 1986 the 7-year-old trees set most fruit (716 fruit per tree), 70 times as many as did the 2-year-old trees (10.7 fruit/tree).

The number of fruit remaining at 50 days after full bloom (final set) was highly influenced by tree age in both years of observation. In 1985, very few fruit (1.8 per tree) were present at final set on the 2-year-old trees. 4-year-old trees had 171 fruit per tree, 6-year-olds had 471 fruit per tree (the highest number in any tree age) and the 12-year-olds were intermediate between these with 255 fruit per tree. Unfortunately, data regarding final set of the 3-year-old trees were unavailable. This was due to the use of these trees by another experimentor, involving the application of chemicals intended to affect tree growth and cropping.

In 1986 the number of fruit present at final set increased with tree age; 2-year-old trees had an average of 8.4 fruit per tree, 3-year-olds 13.9, 5-year-olds 224.7, and 7-year-olds 485.3.

Final set values are determined by the number of fruit buds formed initially, the proportion of these which set fruit, and also by the number of flowers which set initially but subsequently abort and abscise and the number of fruit which abscise later in development. In 1985 no significant differences were observed between the 4-, 6- and 12-year-old trees in terms of the proportions of those fruitlets present at initial set which were then retained until final set assessment. These were in the range of 49%-59%. On the 2-year-old trees however, only 30% of the fruitlets initially set were retained until this time. In all the tree ages examined in

1985, the number of fruits present at final set per 100 fruit clusters was less than 100. On average, more than 50% of clusters on the older trees successfully produced a mature fruit compared with 3.5% on the youngest trees. This difference is the result of several contributory factors. On the youngest trees, the number of fruits set per 100 clusters was lower than on the older trees (14.9% compared to 103-139%); and then a smaller proportion of the fruit set were retained until maturity (30% compared to 49%-59%).

In 1986 the situation was slightly different. Although the numbers of fruit per tree increased steadily with tree age, the factors contributing to this were not of the same pattern as in 1985. Few clusters on the younger trees set fruit initially (21% and 31% on the 2- and 3-year-olds compared to 88% and 91% on the 5- and 7-year-olds respectively). But in contrast to 1985, the younger trees (2- and 3-year-olds) retained a higher proportion of fruitlets between initial set and final set than did older trees.

Seed number in harvested fruit did vary between the various orchards, and in 1985 this appeared to be positively associated with tree age. Fruit from the 2-year-old trees had an average of 5.03 seeds, those from 12-year-old trees 6.84, and those from 4- and 6-year old trees were intermediate. In 1986 this pattern was not retained; fruit from 2-, 3- and 5-year-old trees all had similar numbers of seed within harvested fruit (5.43 on average), those from 7-year-old trees had significantly more at 6.66.

To assess the relative performance of the differently aged trees independently of their physical size, the height and spread of the branch structure of individual trees was measured at both the start and finish of the 1986 season. Tree volume was calculated according to the formula for a cone where $V=^1/_3\pi r^2h$ (where r=tree radius and h=crown height), and then the number of flowers and fruits were calculated per unit volume for each tree age. In April 1986 a thirteen fold difference in tree crown volume was apparent between the youngest and oldest trees, (Table 2.3.1.3), rising from the 0.45 m^3 of the 2-year-olds to the 6.7 m^3 of the 6-year-olds. 4-year-olds were intermediate at 3.93 m^3 per tree and 3-year-old trees had similar volumes to the 2-year-olds.

Calculation of the number of fruit buds produced per $metre^3$ of tree crown volume indicated this to be similar in both the youngest and oldest trees with 78.7 and 77.4 buds per m^3 being produced respectively). The 3- and 4-year-olds produced significantly more than this at approximately 110 buds per m^3.

Similar calculation of the numbers of fruit initially set indicates a different situation. The 5- and 7-year-old trees set approximately 99 and 125 fruit per m^3 respectively but the younger trees set only about 25% this number with between 24 and 29 fruit per m^3.

Tree crown volume of all ages of tree increased during the year. In December 1986 the youngest trees had a volume of 1.44 m^3, the oldest ones 9.46 m^3. Calculation of percentage increase in tree volume indicated significant differences between the different ages of tree.

Table 2.3.1.3. Flower and fruit production in Cox trees of various ages in relation to tree crown volume. Within any row, values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

	age of tree (years)			
	2	3	5	7
tree crown volume (m^3)	0 · 45a	0 · 97b	3 · 95c	6 · 7d
April 1986 (S.E.M.)	(0 · 03)	(0 · 08)	(0 · 38)	(0 · 58)
number of fruit buds/m^3	78 · 7a	105 · 3b	114 · 9b	77 · 4a
(S.E.M.)	(16 · 73)	(13 · 37)	(13 · 40)	(12 · 46)
initial set/m^3	23 · 5a	29 · 3a	99 · 1b	124 · 5c
(S.E.M.)	(8 · 29)	(7 · 40)	(11 · 32)	(13 · 19)
final set/m^3	6 · 28a	8 · 24a	28 · 37b	58 · 47c
(S.E.M.)	(2 · 16)	(3 · 44)	(2 · 76)	(5 · 84)
tree volume (m^3)	1 · 44a	2 · 42b	8 · 37c	9 · 46d
December 1986 (S.E.M.)	(0 · 096)	(0 · 31)	(0 · 71)	(0 · 68)
% increase in tree volume	326 · 8a	259 · 3b	206 · 5c	145 · 6d
April → Dec. 1986 (S.E.M.)	(18 · 3)	(16 · 9)	(10 · 5)	(8 · 6)

The youngest trees had the largest proportional increase in volume (326%), the oldest trees the smallest (146%) and the 3- and 5-year olds intermediate with increases of 259% and 207% respectively.

To assess tree vigour, shoot growth was measured on all trees. In both years the total length of new shoots produced increased with tree age. In 1985 approximately 14 metres of shoots were grown by the youngest trees and 95 metres by the 6-year-old trees. 3- and 4-year old trees were intermediate, having a shoot growth of 43 and 67 metres per tree respectively. Growth on the 12-year-old trees (53 metres per tree) was less than that on the 6-year-olds and more similar to that of the 4-year-olds.

In 1986 2- and 3-year-old trees produced similar amounts of new growth (26.5 and 27.5 metres per tree respectively), but less than that on the 5- and 7-year-olds (71.8 metres and 86.6 metres per tree respectively). Comparing the 2-year-old trees between the years it was seen that those in 1986 produced almost double the shoot growth of those in 1985. In 1985 no differences in the mean length of shoots produced by the various ages of tree were detected (within a range of 28.2 to 37.7 cm. per shoot). In 1986, however, the younger trees grew significantly longer shoots than did the older trees. Mean shoot length on the 2- and 3-year- old trees was 41.4 and 37.2 cm. respectively, whilst on the 5- and 7-year-olds this was 31.6 and 31.3 cm. respectively.

In 1985 the number of shoots per tree increased with tree age, from 2-to 6-years-old. The youngest trees produced 41 shoots per tree, the 6-year-olds 311. 12-year-old trees were again most similar to the 4-year-olds both having about 170 shoots per tree). The 2-year-old trees in 1986 produced significantly more shoots per tree than did the 2-year-olds in 1985 (64 per tree compared to 41) but the 3-year-olds in 1986 produced significantly fewer shoots than did the 3-year-olds in 1985 (74 compared to 152). This indicates that although individual shoot length on the 3-year-olds in 1986 was greater than on the same age of tree in 1985, total growth was lower in comparison because there were fewer shoots.

2.4 Discussion

Three main points regarding the growth and cropping of young trees arise from this study. Firstly, the results confirm what is generally accepted, that young trees grow proportionally more than older ones. In 1986 the youngest tree (2-year-olds) increased their crown volume by 236% whereas the oldest (7-year-olds) increased theirs by only 146%. Within this, the pattern of shoot growth also varied, the youngest trees producing shoots of the longest mean length. This propensity of young trees for vigorous shoot growth, often of relatively few unbranched shoots in the early years is widely recognised (Elfving and Forshey 1976, Tromp and Wertheim 1980, Dennis 1981).

The second point is that flowers were produced on all trees, including the youngest, and when flower numbers were expressed on a per m^3 of crown volume (CV) there were few differences between the various tree ages. The youngest and oldest trees produced very similar numbers of buds per $metre^3$ CV, the two intermediate tree ages both being higher but again very similar to each other. This agrees with the common observation that flowers can be, and often are produced on young trees (Gardner *et al.* 1952, Forshey 1978).

Thirdly, of the flowers on the young trees, very few were successfully converted into fruitlets. In both years the percentage fruit set was very low on these trees, being 30% and 20% for the 2-year-old trees of each year. This was reflected in the number of fruit set per m^3 CV where on the 2- and 3-year- old trees only 23 and 29 fruitlets/m^3 were set, compared to 99 and 124 fruitlets/m^3 on the 5- and 7-year-old trees respectively. The fact that the number of fruits initially set per m^3 CV on 2- and 3-year-old trees was much lower than on 5- and 7-year-old trees, despite the similar numbers of fruit buds per m^3 CV in the youngest and oldest trees and in 3- and 5-year-old trees, suggest that the ability of fruit buds to set fruit may constitute one component of the cropping problem within young orchards. Indeed, the 3-year-old trees, although having significantly more fruit buds per $metre^3$ CV than the 7-year-old trees, produced only about $^1/_4$ of the number of fruit initially set/m^3. The fact that final set numbers were generally reduced by a similar proportion on all tree ages suggests that the main factor contributing to cropping differences between the various tree ages was neither the number of flowers produced nor the proportion of fruitlets retained throughout development but rather was due to lack of flowers setting fruit initially. However, it is of interest to note that in 1986 the 7-year-old trees retained a slightly higher proportion of fruits initially set and also their fruits had more seeds than was tha case with any other age of tree. The number of seeds within a fruit are the product of the number of ovules successfully fertilised minus the number of embryos aborted during development. Because seeds are a rich source of gibberellin (Dennis and Nitsch 1966) and auxin (Crane 1964, 1969) they maintain metabolic activity within the fruitlet, allowing it to attract nutrients, and consequently, grow. It is suggested that the more seeds contained in a fruit, the better it is able to withstand competition both from the vegetative parts of the tree and also from other fruitlets (Abbott 1984). The higher seed number within fruit from 7-year-old trees may therefore suggest that ovule fertilisation had been more successful consequently conferring a greater ability to compete with shoot growth with the result that a higher proportion of fruits initially set were retained until final set assessment. As Abbott (1984) pointed out, it is not the absolute number of seeds within a fruit which determines whether or not it will be retained until maturity - fruits with one seed can remain attached, and continue to grow as well as those with ten. What is important is the number of seeds relative to the number of nearby fruitlets and to the availability of resources.

Dennis (1981) suggested that initial set is the main determinant of crop yield in mature trees and also pointed out that although blossom density is not unimportant in cropping, it is less likely to limit yield than is fruit set. However, this is not always the case. Luckwill and Child (1973) found that in highly intensive systems, flowering and a low June drop were the most important factors contributing to high levels of cropping. Although these conclusions are apparently contradictory, this may simply reflect the differences between high and low density fruit production systems.

If fruit set is the major factor limiting yield in young Cox orchards, then what are the possible reasons underlying it, and what factors influence fruit set?

The most obvious factor that comes to mind is that of pollination. In order for Cox flowers to set fruit they must be pollinated with compatible pollen during the period when they are receptive. If the young orchards had either insufficient pollinators present, or a poor supply of compatible pollen, then lack of fertilisation, and subsequently, poor fruit set, would be an inevitable consequence. However, all the Cox trees used were within orchards containing a good proportion of suitable pollinating varieties, and all had hives of bees inserted during the flowering period. This of course still does not guarantee pollination; bees may either not fly due to adverse weather, may be attracted to other nearby crops, or the pollen of the other varieties may not be viable. However, in each orchard examined, fruit set arising from hand pollinations was also assessed (see Chapter 5) and the results virtually mirrored those of the open pollinations. Thus we can assume that the flowers of all orchards were adequately pollinated, and that the observed differences in set between them must reflect some differences within the trees or their flowers that influences their ability to set fruit.

However, although fruit set is dependent on pollination and fertilisation, it is also influenced by the 'strength' or 'quality' of the blossom. Floral 'strength' is a widely used expression to describe the probability of a flower becoming a fruitlet following pollination with compatible pollen during its receptive period (May 1972) but which has never been satisfactorily understood or defined. Intrinsically the floral 'strength' must be a function of flowers with mature and correctly formed components such that pollen can germinate on the stigma, grow through the style and reach ovaries which have the correct spatial arrangement of nuclei and remain meristematically active. For this state to be reached in a flower, and in order to supply photosynthate to the developing fruitlet, clusters must have an adequate, physiologically active leaf area plus good vascular connections to the main tree allowing nutrients to flow freely from there to the flower. In addition the degree of competition exerted by the rapidly growing shoot tips must not be so strong that the majority of available nutrients are drawn to them, leaving insufficient for the flowers and fruitlets.

Floral 'quality' is often assessed visually in terms of flower and/or leaf size and/or colouration (Williams 1965, Goldwin 1978, Abbott 1984) and authors have reported that 'weak' blossom

may be formed on trees with vigorous growth (Hill-Cottingham and Williams 1967), trees which had borne a heavy crop the previous year (Buszard 1983), and trees subjected to warm spring temperatures (Miller 1988). Conversely 'strong' or high 'quality' flowers have been formed following summer nitrogen application (Williams 1965, Hill-Cottingham and Williams 1967), defruiting (Buszard 1983) and where flowers had been initiated early in the preceding season (Abbott 1970).

Of the above situations associated with variable flower 'quality', two can probably be discounted as being unlikely to have contributed to the different levels of fruit set obtained in the various orchards examined here. Firstly although due to the amount of available shelter, and the slight delay in flowering of the youngest trees in 1985 there may have been small differences in climate between the various orchards during flowering, spring temperatures would have been very similar within all the trees. Secondly, previous crop load (with which flower 'quality' varies inversely) was (certainly in 1986, and presumably in 1985) higher on the older trees compared to the younger ones.

This suggests that the poor fruit set obtained on the younger trees may have been due to one of three things. Firstly the buds on the younger trees may have been initiated late during the preceding season and consequently developed less far prior to dormancy than had buds on the older trees. Alternatively, initiation may have occured at the same time on all tree ages but fewer nutrients were directed towards those on the young tree such that they had lower reserves to draw upon the following spring. Both of these possibilities tie in with the observation that the younger trees had relatively higher levels of shoot growth than the older trees. Although no measure of shoot growth kinetics was made during the season, measurements the following year showed that growth on young trees continued later than it did on older trees (Chapter 7). Since flower initiation is often reported to occur around the time of shoot growth cessation (Barnard and Read 1932, Huang 1987) this extended growth period could induce 'young buds'; alternatively if initiation occured at the same time on all tree ages then presumably the extended growth on the young trees would have reduced the amount of assimilate available to each developing bud.

Secondly, as regards the effect of nitrogen fertiliser, although all orchards were maintained by the same management programme, it is obviously possible that the level of available nutrition could vary between orchards. Thus the younger trees might have been hindered by a lack of an adequate supply of essential nutrients. Additionally differences in their roots - either in pattern, amount or growth could also affect vegetative growth and cropping.

Thirdly it is possible that in addition to continuing growth until later in the season, shoot growth on young trees may commence earlier. That shoot growth is inhibitory to fruit set has been shown very clearly by Abbott (1960) and Quinlan and Preston (1971). Thus if shoots on young trees start to grow before those on older trees, or grow more strongly initially, during the time

of flowering/early fruitlet development, then metabolites will be attracted towards them, leaving less available for the flowers which will then have to compete very strongly.

The question of what limits fruit set in young trees is investigated in more detail in the remaining chapters.

Chapter 3. Influence of plant growth regulators, shoot tipping, branch orientation and tree age on shoot growth, fruit bud number and fruit set.

3.1 Introduction.

High, consistent fruit yields are the aim of any orchard. Within newly established low density plantings, vegetative growth is also needed. Because there is a reported antagonism between growth and fruit production (Lang 1961) it might be assumed that one would always be at the expense of the other. Indeed, the rapid shoot growth which can occur during the early years of orchard establishment has been seen to reduce both flower production (Batjer *et al.* 1963, Mika *et al.*1977) and fruit set (Abbott 1960, Quinlan and Preston 1971, Hansen 1980). However, relatively little work has been done to investigate methods which might improve the cropping of young trees whilst also allowing shoot growth to continue. The possibility of achieving this may be there, but it is as yet unrealised. Within mature trees there are several methods of increasing flower and fruit production. However, often these also decrease shoot growth. Applied indiscriminately to a young tree, they may indeed induce earlier cropping, but they may also adversely affect the general structure of the tree. The early growth and lateral branching of young apple trees is an important factor in inducing early fruit production (Avery 1969), increasing the trees' fruiting surface and early cropping potential (Kender and Carpenter 1972, Sheperd 1979) therefore it is important that this is not detrimentally affected by treatments which are applied to improve cropping.

Techniques such as root pruning, scoring, girding and tying down branches are long established practices used to encourage orchard productivity.

Scoring and girdling are essentially similar techniques - with scoring, an inscision is made through the bark of the trunk or branch, with girdling a complete band of bark is removed. Both techniques cut through the phloem tissue and effectively interrupt the basipetal translocation of carbohydrates and growth regulators as they move from the leaves towards the roots. This retains them within the upper part of the tree where they can provide enriched resources for developing flowers and fruits. From MacDaniels and Heiniche (1930) reporting that ringing in the spring increased fruit set to numerous more recent papers (see below), the evidence points to them being very successful techniques for increasing fruit numbers.

By scoring trunks of 4-year-old 'Red Prince' and 'Melrose' apple trees Stang *et al.* (1976) increased the number of flowers per tree from 9 and 159 on controls to 111 and 343 respectively. Similarly, by scoring the trunks of 7-year-old 'Puritan' trees 3-4 weeks after full bloom Southwick *et al.* (1967) increased the number of flowers and fruit the following year by 500% compared to controls.

Another long established techique for improving orchard productivity is that of training upright branches to a more horizontal position (Langley 1729, Knight 1803, Preston 1974). It too has been shown to increase carbohydrate content within treated branches (Kato and Ito 1962)

but experimental investigation into its effects on flower and fruit production have given variable results.

Wareing and Nasr (1958, 1961) clearly showed that in controlled conditions, a horizontal apple rootstock produced more flowers the following year than did an equivalent vertical one. Similarly Tromp (1970) trained branches of 1-year-old 'Golden Delicious' trees to a horizontal position and increased the number of good quality flower buds from 23 per tree to 50 per tree.

In contrast, both Mika (1969) and Greene and Lord (1978) found no increase in bud numbers associated with a horizontal branch orientation.

However, within all these experiments, concurrent with any increased flower number, vegetative growth was reduced - usually by about 1/3 compared to equivalent upright branches and thus they would not have been entirely satisfactory for use on young, growing trees.

One disadvantage of cultural methods such as these is that they are very labour intensive, and as such, expensive. Consequently the possibility of acheiving the same effects through the use of apparently much more convenient chemical sprays has been met with much enthusiasm.

Since the early experiments with daminozide demonstrated its ability to improve flower and fruit production (Batjer *et al.* 1964), it, along with several other chemicals such as ethrel and paclobutrazol have been widely tested and used in efforts to improve productivity.

Generally this is successful, Williams and Thompson (1979) found that 2000 ppm daminozide in combination with phosphate applied at planting increased the number of flower buds per tree from 13.5 on controls to 46.5, and doubled the numbers of harvestable fruit. Tukey (1983) increased the yield of bearing trees 3 fold by application of 1000-2000 ppm paclobutrazol the year before and Quinlan *et al.* (1985) using 2000 ppm paclobutrazol increased the numbers of flowers and fruit on 5-year-old Cox/MM106 5 fold.

Greene and Lord (1978) found that ethrel applied at various rates (500-1000 ppm) 2 weeks after full bloom to increase the number of flowers initiated 2 years out ot 3 but that this increased bloom was not always reflected in increased numbers of fruits set.

Again though, as with the cultural techniques, vegetative growth was decreased by all these treatments. Therefore although several ways of attempting to increase fruit buds number exist, they also have the potential to decrease vegetative growth.

Thus a situation exists where (a), due to lack of flowers and/or fruit set, young orchards remain unfruitful for several years, whilst (b), there are several known methods for increasing the flower and fruit production of older trees. It would seem sensible that in order to try and overcome 'a', the effectiveness and suitability of the methods and techniques in 'b' should be investigated within young trees.

However, there is an alternative approach to the problem of low production from young orchards. Rather than concentrating on increasing the fruiting of young trees, by attempting to bring young trees rapidly into bearing, they could perhaps be encouraged to grow faster initially such that allocated orchard space was filled more quickly. There are theories that in order to reach maturity, bear flowers and fruit, a tree must first reach a certain size (Zimmerman 1971). If this is true and is reflected in the time taken for clonally propagated material to 'age' and produce fruit, then an initially faster rate of vegetative growth may reduce the duration of the non-fruiting stage.

Phosphorus application at planting can increase the growth of young trees (Taylor 1975) and gibberellin application is widely reported to stimulate shoot production (Greenhalgh and Edgerton 1967, Bangerth *et al.* 1986) although usually it also decreases flower production (Dennis and Edgerton 1966, Tromp 1987).

Bearing all these facts in mind, the aim of the experiments described in this chapter was firstly to evaluate the potential of some of the methods commonly used to increase fruiting in mature trees by assessing their effects on young trees. The second aim was to investigate the effects of gibberellin application on the growth and fruiting of young trees.

3.2 Materials and methods

3.2.1 Experiment 1:

Effect of Gibberellic acid (GA_3) on shoot growth and fruit-bud formation.

One-year-old Cox trees, on both M9 and MM106 rootstocks were treated with 5, 50, or 500 ppm GA_3 (applied as the commercial preparation Berelex) on either April 12th, May 12th or June 15th 1984. Whole trees were sprayed to incipient run-off using a hand gun sprayer; control trees were unsprayed. Tween 20 at a concentration of 1000 ppm was used as surfactant. All treatments were applied to eight single tree replicates within randomised block designs for each rootstock. Shoot growth was measured in December and the number of fruit buds per tree counted in April 1985.

3.2.2 Experiment 2:

Effect of plant growth regulators, shoot tipping and branch-bending on shoot growth, fruit-bud formation and fruit set in young trees

Two-year-old defruited trees of Cox on M9 rootstock, and Bramley's Seedling (Bramley) on M26 rootstock were treated as follows:

(i) 1000 ppm daminozide (Alar from Dow) with 1000 ppm Tween 20 as surfactant, applied to incipient runoff using a hand gun sprayer,

(ii) 1000 ppm paclobutrazol (PP333 from I.C.I.) applied as above,

(iii) All extension shoot tips removed,

(iv) All branches and extension shoots tied/weighted down to a horizontal position.

Treatments (i) - (iii) were also applied to defruited Cox trees on MM106 rootstock. Treatments were applied once, on either June 16th or August 15th 1985 to eight (Cox) or 20 (Bramley) single tree replicates within randomised block designs. Control trees were untreated. Shoot growth was measured in November 1985 and 1986, the number of fruit buds produced, their initial and final set, and harvested fruit seed number were counted in 1986.

3.2.3 Experiment 3:

Effect of branch angle and tree age on shoot growth and fruit-bud formation in young trees.

This experiment started in 1984, was run for 3 years. Each year shoot growth, fruit-bud number, initial and final fruitset were measured. In the first year (1984), shoots of newly planted Cox on both MM106 and M9 were trained to a vertical, horizontal or intermediate (45°) orientation. Downward pressure from string attached to pegs in the ground was used to increase branch angle, electrical insulating tape holding branches close to the trunk was used to narrow it.

Trees were blocked by girth and treatments were applied to 24 or 36 single tree replicates within a randomised block design with 2 or 3 replicates per block respectively. In 1984 there were 24 replicates of treatments where branches were trained to horizontal or 45°, and 36 replicates of trees with branches trained to a vertical position. In 1985 and 1986 treatments were reapplied to all trees. However, the 'horizontally trained' treatment was subdivided. Within each block, half of the trees designated to have horizontally trained branches had branches retied to a horizontal position at the start of the season but were then left to grow without interference; the remainder had branches similarly retrained at the start of the season but were then monitored weekly, the weights used to train the shoots being moved towards the shoot tip such that shoot tips remained horizontal throughout. The treatments were continued in 1986.

3.2.4 Experiment 4:

Effect of altering branch angle, either during or after seasonal growth, on fruitlet retention, shoot growth, fruit-bud formation and fruit set.

Branches of 3-year-old untrained Discovery on MM106 rootstock were tied down to a horizontal position either at one of three times during the growing season, or after vegetative growth had stopped. These times corresponded to two weeks before, 4, 12 or 21 weeks after full bloom. Trees were blocked by fruit bud number, and treatments were applied to 11 single tree replicates within a randomised block design.

Initial fruit set was assessed three weeks after full bloom; when fruitlets began to abscise, the fallen fruitlets were collected from under each tree, counted and individually weighed. Collections of fallen fruit were made at 3-5 day intervals throughout the period of 'June Drop' (25 June - 10 July).

Shoot growth was measured in December 1984, and the number of fruit buds produced per tree and initial and final fruit set assessed in 1985.

Although not accounted for in the original experimental design, it was later considered desirable to include trees, the branches of which had remained vertical throughout the winter of 1984/1985 in an assessment of bud numbers in 1985. To provide this informal control, trees from the remainder of the plot were used. Although the original design used only trees from one area of the plot, buds had been counted on all trees in spring 1984. These counts enabled selection of trees, the bud numbers of which were comparable to each of the 11 original blocks.

3.3. Results

3.3.1. Experiment 1:

Effect of Gibberellic acid (GA_3) on shoot growth and fruit-bud formation.

Analysis of the shoot growth arising in 1984, and the number of fruit buds produced in 1985 indicated gibberellin to have a differential response dependent on which rootstock the trees were grown on. Within trees on MM106 rootstock, application of either 5, 50, or 500 ppm GA_3 in either April, May or June did not significantly alter either the total shoot growth or the mean shoot length produced (Tables 3.3.1.1a and 3.3.1.2a). Control trees produced 524 cm. of new shoots, with a mean length of 29.9 cm. Within the treated trees, these values were within a range of 457 to 582 cm. and 28.4 to 38.1 cm. respectively. Variation within treatments was high, and no differences between treatments were detected.

Of trees on M9 rootstock, controls produced 388.8 cm. of new shoots. May application of 50 ppm GA_3 significantly reduced this to 291.0 cm. (Table 3.3.1.1b). Mean shoot length on the control trees was 44.7 cm.; that on the treated trees was within a range of 35.5 to 49.5 cm. and appeared unaffected by any treatment (Table 3.3.1.2b). Although total shoot growth produced during 1984 was much higher within the trees on MM106 compared those on M9, mean shoot length was greater on the latter.

The number of fruit buds produced in 1985 by trees on MM106 were not influenced by GA_3 application. Control trees produced an average of 67 buds/tree; all GA_3 treatments resulted in a lower average value than this (in a range of 36-62 buds/tree) but variation within treatments was again high and differences were not significant (Table 3.3.1.3a).

Within trees on M9 rootstock, fruit bud numbers generally were much lower than were seen within the trees on MM106 and were significantly affected by gibberellin application. Control trees produced an average of 10.2 fruit buds each (Table 3.3.1.3b). Trees sprayed in May however, generally produced more buds than this. Although not significant within each treat-

Tables 3.3.1.1a + b. Total length of shoots (cm.) produced by Cox trees on (a) MM106 and (b) M9 rootstocks when treated with GA_3. Trees were sprayed with 5, 50, or 500 ppm (active ingredient) GA_3 in either April, May or June. Control trees were unsprayed. S.E.D.'s and significance levels shown are for comparison with controls. * indicates significance at the $P \leqslant 0 \cdot 05$ level.

(a) = trees on MM106 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	457	468	335	487
50	461	464	460	462
500	577	582	516	558
control				524
date mean	498	505	504	

S.E.D.: Treatment × Date = 117 · 4; Dates = 95 · 9; Treatments = 95 · 9.

(b) = trees on M9 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	414	417	354	395
50	410	291*	400	367
500	386	347	455	396
control				389
date mean	403	352	403	

S.E.D.: Treatment × Date = 40 · 9; Dates = 35 · 0; Treatments = 35 · 0.

Tables 3.3.1.2a + b. Mean shoot length (cm.) of Cox trees on (a) MM106 and (b) M9 rootstocks when treated with GA_3. Trees were sprayed with 5, 50, or 500 ppm (active ingredient) GA_3 in either April, May or June. Control trees were unsprayed. S.E.D.'s shown are for comparison with controls.

(a) = trees on MM106 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	28 · 4	29 · 7	31 · 2	29 · 8
50	28 · 0	28 · 7	35 · 9	27 · 5
500	34 · 5	38 · 1	29 · 5	34 · 0
control				29 · 9
date mean	30 · 3	32 · 1	28 · 9	

S.E.D.: Treatment × Date = 6 · 52; Dates = 5 · 32; Treatments = 5 · 32.

(b) = trees on M9 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	40 · 5	39 · 3	35 · 7	38 · 5
50	41 · 9	35 · 5	49 · 5	42 · 3
500	35 · 8	38 · 3	44 · 2	39 · 3
control				44 · 7
date mean	39 · 4	37 · 7	43 · 2	

S.E.D.: Treatment × Date = 5 · 21; Dates = 4 · 25; Treatments = 4 · 27.

Tables 3.3.1.3a + b. Number of spur fruit buds (under $\sqrt{\ }$ transformation) produced by Cox trees as (a) MM106 and (b) M9 rootstocks when treated with GA_3. Trees were sprayed with 5, 50, or 500 ppm (active ingredient) GA_3 in either April, May or June. Control trees were unsprayed. S.E.D.'s and significance levels shown are for comparison with controls. * indicates significance at the $P \leqslant 0 \cdot 05$ level.

(a) = trees on MM106 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	7 · 00	7 · 37	6 · 53	6 · 97
50	7 · 84	7 · 01	7 · 69	7 · 51
500	6 · 80	6 · 01	7 · 94	6 · 92
control				8 · 14
date mean	7 · 21	6 · 80	7 · 39	

S.E.D.: Treatment × Date = 0 · 966; Dates = 0 · 788; Treatments = 0 · 788.

(b) = trees on M9 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	3 · 47	4 · 25	3 · 78	3 · 83
50	3 · 96	4 · 57	3 · 06	3 · 86
500	3 · 82	4 · 40	3 · 07	3 · 77
control				3 · 19
date mean	3 · 75	4 · 41*	3 · 30	

S.E.D.: Treatment × Date = 0 · 602; Dates = 0 · 493; Treatments = 0 · 491.

ment level, when these were combined, (giving a mean of 19.4 buds/tree) the differences were significant.

Although Cox rarely produces fruit on one-year-old wood, flowers are frequently formed there, often abscinding without forming fruitlets. Counts of these flowers showed gibberellin to have significantly influenced their production within trees on both rootstocks. Within trees on MM106, application of 500 ppm GA_3 in May decreased the number of axillary buds formed twenty fold (Table 3.3.1.4a). Within the trees on M9 rootstock, the number of axillaries formed was was consistently lower than on Cox/MM106. In contrast to the situation within Cox/MM106, June application GA_3 had greater effect on axillary bud production than did April or May applications (Table 3.3.1.4b). Control trees averaged 10 axillaries per tree; this was reduced to less than one per tree by June application of GA_3.

3.3.2. Experiment 2:

Effect of plant growth regulators, shoot tipping and branch bending on shoot growth, fruit-bud formation and fruit set in young trees.

Within all three cultivar/rootstock combinations (Bramley/M26, Cox/M9 and Cox/MM106) some of the applied treatments affected aspects of shoot production either during the year of treatment application and/or the one following. In addition, some treatments affected the components of fruit production during the year following treatment. Often the two types of response were seen together. Treatment effects within each cultivar/rootstock combination are reported separately in the following sections, and the regression analyses for shoot growth characters against components of fruit production are presented collectively at the end.

3.3.2.1 Bramley on M26 Rootstock.

Shoot growth in 1985 was markedly affected by some of the treatments imposed. Although shoot number was unaffected by treatment (Table 3.3.2.1a), mean shoot length was decreased by June applications of paclobutrazol or daminozide, and increased by shoot-tipping in June (Table 3.3.2.1b). Compared to control trees, paclobutrazol and daminozide reduced mean shoot length by about $^1/_3$, and shoot-tipping increased it by 18%. These changes were partly reflected in the amount of total growth produced in 1985; paclobutrazol and daminozide decreased this by about 43% and 34% respectively, but shoot-tipping increased it significantly (Table 3.3.2.1c). Although no significant differences in shoot number or mean shoot length had been detected on trees where branches had been tied into a horizontal position, total shoot growth was decreased by about 15% compared to controls by this treatment applied in June.

During 1986 it was apparent that some of the treatments applied in 1985 were continuing to affect shoot growth. This was most pronounced in trees which had been treated with paclobutrazol, within which both shoot number and individual shoot length were severely re-

Tables 3.3.1.4a + b. Number of axillary fruit buds (under $\sqrt{}$ transformation) produced by Cox trees as (a) MM106 and (b) M9 rootstocks when treated with GA_3 Trees were sprayed with 5, 50, or 500 ppm (active ingredient) GA_3 in either April, May or June. Control trees were unsprayed. S.E.D.'s and significance levels shown are for comparison with controls. *, ** indicates significance at the $P \leqslant 0 \cdot 05$ or 0.01level respectively.

(a) = trees on MM106 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	4 · 70	5 · 59	5 · 21	5 · 17
50	3 · 88	3 · 86	4 · 96	4 · 23
500	4 · 41	0 · 90*	5 · 21	3 · 51
control				4 · 76
date mean	4 · 33	3 · 45	5 · 13	

S.E.D.: Treatment × Date = 1 · 146; Dates = 0 · 936; Treatments = 0 · 937.

(b) = trees on M9 rootstock

GA_3 concentration	date of application			treatment
(ppm)	April	May	June	mean
5	1 · 33	1 · 94	0 · 92*	1 · 40
50	3 · 08	2 · 24	1 · 01	2 · 11
500	2 · 58	3 · 28	0 · 78*	2 · 22
control				3 · 19
date mean	2 · 33	2 · 49	0 · 90**	

S.E.D.: Treatment × Date = 0 · 937; Dates = 0 · 770; Treatments = 0 · 766.

Tables 3.3.2.1a-l Components of shoot growth and fruit production in Bramley apple trees treated with Paclobutrazol, Daminozide, shoot tipping or tying branches to a horizontal position. Treatments were applied once, either in June or August 1985, shoot growth was measured in 1985 and 1986; components of fruit production were assessed in 1986. S.E.D. and significance levels refer to comparison with control (n=8). *, **, ***, indicate significance at the $P \leqslant 0 \cdot 05$, $0 \cdot 01$ or $0 \cdot 001$ levels respectively.

Tables are:-

(a) shoot number 1985
(b) mean shoot length 1985
(c) total shoot growth 1985
(d) shoot number 1986
(e) mean shoot length 1986
(f) total shoot growth 1986
(g) number of spur buds produced in 1986
(h) number of fruit set per 100 flower clusters
(i) initial set of spur fruit buds 1986
(j) percentage of initial set retained until final set assessment
(k) final set of spur fruit buds 1986
(l) seed number in harvested fruit

Table 3.3.2.1 (legend on facing page)

(a) = shoot number 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	16 · 4	16 · 8	16 · 6
Daminozide	17 · 6	16 · 7	16 · 9
Tipped	16 · 2	15 · 0	15 · 6
Tied	16 · 3	17 · 7	17 · 0
Control			17 · 7
Date mean	16 · 5	16 · 5	

S.E.D.: Treatment × Date = 1 · 85; Dates = 1 · 02; Treatments = 0 · 94.

(b) = mean shoot length (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	28 · 2***	43 · 0	35 · 6**
Daminozide	31 · 7***	44 · 1	37 · 9*
Tipped	53 · 7*	51 · 0	52 · 3*
Tied	43 · 1	46 · 4	44 · 8
Control			45 · 5
Date mean	39 · 2***	46 · 1	

S.E.D.: Treatment × Date = 3 · 44; Dates = 2 · 72; Treatments = 2 · 98.

(c) = total shoot growth (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	460***	700	580***
Daminozide	527***	720	624**
Tipped	873	741	807
Tied	683*	790	736
Control			806
Date mean	636***	738	

S.E.D.: Treatment × Date = 58 · 5; Dates = 46 · 3; Treatments = 50 · 7.

(d) = shoot number 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	4 · 2***	6 · 9***	5 · 5***
Daminozide	55 · 5	54 · 3	54 · 9
Tipped	50 · 1	49 · 5	49 · 8
Tied	50 · 7	48 · 4	49 · 6
Control			52 · 3
Date mean	40 · 1*	39 · 8*	

S.E.D.: Treatment × Date = 6 · 94; Dates = 5 · 48; Treatments = 6 · 01.

stricted (Tables 3.3.2.1d and e). Averaged over the two application times, paclobutrazol reduced shoot number from 52.3 per tree on controls to only 5.5 per tree. Simultaneously individual shoot length was decreased from an average of 48.9 cm. on controls to 14.2 cm. These two factors combined to reduce the total shoot produced on these trees to only about 5% of that produced on the controls (Table 3.3.2.1f).

Thus some of the treatments affected shoot growth during the year that the treatments were applied and/or the one following. There are many references in the literature suggesting that shoot vigour and flower initiation are inversely related (Lang 1961, Monselise and Luckwill 1974, Williams 1983) and also that flowers and/or developing fruits have to compete with the vegetative part of the tree for nutrients (Abbott 1960, Quinlan and Preston 1971). Bearing this in mind, it might be anticipated that in addition to influencing shoot growth, the treatments applied in 1985 may also have influenced flower initiation and/or fruitlet set and retention. Analysis showed this to be the case.

The number of fruit buds produced in 1986, and the proportion of these which set fruit were variously affected by the different treatments. However, this was not restricted to the treatments where significant differences in shoot growth characteristics had been found.

When applied in June, paclobutrazol, daminozide and tying down branches all increased the number of spur fruit buds formed compared to the controls. August application of paclobutrazol also did this (Table 3.3.2.1g). These treatments raised fruit bud number from 15.7 per tree on controls to 30, 23, 29 and 23 respectively.

Calculation of the number of fruitlets set per flower cluster showed that paclobutrazol had increased this but that shoot-tipping or branch tying in June had decreased it (Table 3.3.2.1h). Almost half of the flower clusters on paclobutrazol treated trees successfully set a fruitlet, whereas only approximately 20% of clusters on control trees, and 5% of those within trees where shoot-tips had been removed or branches tied down in June did. Combining these results with those concerning the number of flower buds produced per tree suggests that the number of fruit initially set per tree was likely to be a factor greatly affected by the paclobutrazol treatments. This was indeed the case; the average number of fruits set on paclobutrazol treated trees was more than 10 times the number set on control trees (Table 3.3.2.1i). Although tying branches to a horizontal position had increased the number of fruit buds produced, the decreased percentage of these which set resulted in the trees given this treatment being very similar to the control trees in the numbers of fruits initially set. No differences associated with any other treatment were seen. Seed number did not vary significantly between the treatments, fruits had an average of 6.35 seeds each (Table 3.3.2.1l).

Although initial set is thought to be the most important factor governing yield (Dennis 1981), fruits must of course remain on the tree until maturity if they are to contribute to yield. Thus any way in which treatments affect fruitlet retention will also affect yield. However, only one

Table 3.3.2.1 continued

(e) = mean shoot length (cm.) 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	13 · 3***	15 · 2***	14 · 2***
Daminozide	43 · 3	42 · 0	42 · 6
Tipped	46 · 0	45 · 3	45 · 6
Tied	41 · 9	46 · 3	44 · 1
Control			48 · 9
Date mean	36 · 1**	37 · 2**	

S.E.D.: Treatment × Date = 4 · 34; Dates = 3 · 43; Treatments = 3 · 76.

(f) = total shoot growth (cm.) 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	69***	107***	88***
Daminozide	2430	2147	2289
Tipped	2129	2247	2188
Tied	2119	2256	2187
Control			2561
Date mean	1688*	1688*	

S.E.D.: Treatment × Date = 278 · 2; Dates = 219 · 9; Treatments = 240 · 9

(g) = number of spur fruit buds produced 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	5 · 46***	4 · 77*	5 · 11*
Daminozide	4 · 77*	4 · 44	4 · 61
Tipped	3 · 23	3 · 73	3 · 48
Tied	5 · 35**	3 · 26	3 · 40
Control			3 · 96
Date mean	4 · 25	4 · 05	

S.E.D.: Treatment × Date = 0 · 366; Dates = 0 · 317; Treatments = 0 · 290

(h) = number of fruit set per 100 flower clusters

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	47 · 8**	34 · 7	41 · 2**
Daminozide	6 · 8	7 · 4	7 · 1
Tipped	3 · 4*	26 · 1	14 · 7
Tied	5 · 5*	13 · 7	9 · 6
Control			20 · 6
Date mean	15 · 9	20 · 5	

S.E.D.: Treatment × Date = 7 · 12; Dates = 6 · 17; Treatments = 5 · 63

Table 3.3.2.1 continued

(i) = initial set of spur fruit buds (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	3 · 89***	2 · 87***	3 · 38***
Daminozide	1 · 23	1 · 26	1 · 25
Tipped	0 · 20	0 · 14	0 · 17
Tied	0 · 80	0 · 67	0 · 73
Control			0 · 89
Date mean	1 · 53	1 · 24	

S.E.D.: Treatment × Date = 0 · 478; Dates = 0 · 378; Treatments = 0 · 414.

(j) = percentage of initial set retained until final set assessment

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	36 · 0	60 · 8	48 · 4
Daminozide	37 · 1	49 · 4	43 · 3
Tipped	57 · 6	27 · 6*	42 · 6
Tied	56 · 0	81 · 8	68 · 9
Control			63 · 9
Date mean	46 · 7	54 · 9	

S.E.D.: Treatment × Date = 14 · 0; Dates = 12 · 1; Treatments = 11 · 07

(k) = final set of spur fruit buds (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	2 · 293***	2 · 474***	2 · 386***
Daminozide	0 · 764	0 · 790	0 · 777
Tipped	0 · 766	0 · 596	0 · 681
Tied	0 · 979	1 · 256	1 · 117
Control			0 · 943
Date mean	1 · 201	1 · 279	

S.E.D.: Treatment × Date = 0 · 276; Dates = 0 · 242; Treatments = 0 · 221

(l) = seed number in harvested fruit

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	6 · 48	6 · 53	6 · 51
Daminozide	6 · 51	6 · 49	6 · 50
Tipped	6 · 01	6 · 11	6 · 06
Tied	6 · 34	6 · 29	6 · 32
Control			6 · 41
Date mean	6 · 34	6 · 35	

S.E.D.: Treatment × Date = 0 · 32; Dates = 0 · 26; Treatments = 0 · 27

treatment was seen to influence the proportion of initially set fruits which were retained until assessment of final set (at which time, fruits would be expected to remain on the tree until harvest). On trees where shoot tips had been removed in August, only 27% of initially set fruitlets were retained until final set. On control trees 64% were retained (Table 3.3.2.1j). However, this did not give rise to any significant differences in the absolute number of fruit present at final set assessment. The only treatments to affect this was paclobutrazol, which increased the absolute number of fruit at final set assessment irrespective of application date (Table 3.3.2.1k). This was due to a combination of a higher number of fruit buds being formed and a higher proportion of these setting fruit.

3.3.2.2 Cox on M9 rootstock

In general, Cox on M9 rootstock were much less affected by the applied treatments than were the Bramley. No component of shoot growth was significantly affected during the year of treatment (Tables 3.3.2.2a - c), and in 1986, the year following treatment, only daminozide application was associated with any significant change in shoot growth (Tables 3.3.2.2d - f). Both times of daminozide application were associated with a reduction in the number of shoots per tree, from 30.9 on control trees to 13.1 and 10.4 on the June and August treated trees respectively (Table 3.3.2.2e). August daminozide application was also associated with a decrease in individual shoot length, reducing it to less than half that found on the control trees (3.3.2.2f). The net result of these effects was that the reduction in shoot number associated with June daminozide application did not reduce the total shoot growth significantly whereas the combination of reduced shoot number and reduced shoot length associated with the August daminozide treatment did (3.3.2.2d).

Compared to controls, 1986 fruit bud number was more than doubled by the June applications of either daminozide or paclobutrazol (Table 3.3.2.2g). Although the August daminozide application greatly increased the number of fruits set per cluster and was the only treatment to do so (Table 3.3.2.2h), only the earlier applications of daminozide or paclobutrazol were associated with an increase in the absolute number of fruits initially set (Table 3.3.2.2i).

As mentioned in relation to Bramley, although highly affected by initial set, yield is also influenced by the proportion of initially set fruits which are retained until harvest. Within Bramley, this had been reduced by removal of shoot tips in August, but within Cox/M9, the same treatment applied in June increased it. Compared to controls, two times as many initially set fruitlets were retained (Table 3.3.2.2j). August daminozide applications also increased the proportion of initial set retained until final set by the same amount.

However, although paclobutrazol application in August had increased the number of fruits set per cluster, and tipping in June had increased the proportion of initial set retained until final set, analysis of the number of fruit present at final set showed that only paclobutrazol ap-

Tables 3.3.2.2a-l Components of shoot growth and fruit production in Cox trees (on M9 rootstock) treated with Paclobutrazol, Daminozide, shoot tipping or tying branches to a horizontal position. Treatments were applied once, either in June or August 1985, shoot growth was measured in 1985 and 1986; components of fruit production were assessed in 1986. S.E.D. and significance levels refer to comparison with control (n=8). *, **, ***, indicate significance at the $P \leqslant 0 \cdot 05$, $0 \cdot 01$ or $0 \cdot 001$ levels respectively.

Tables are:-

(a) shoot number 1985
(b) mean shoot length 1985
(c) total shoot growth 1985
(d) shoot number 1986
(e) mean shoot length 1986
(f) total shoot growth 1986
(g) number of spur buds produced in 1986
(h) number of fruit set per 100 flower clusters
(i) initial set of spur fruit buds 1986
(j) percentage of initial set retained until final set assessment
(k) final set of spur fruit buds 1986
(l) seed number in harvested fruit

Table 3.3.2.2 (legend on facing page)

(a) = shoot number 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	25 · 9	25 · 3	25 · 6
Daminozide	25 · 0	27 · 6	26 · 3
Tipped	29 · 5	27 · 4	28 · 4
Tied	29 · 3	30 · 0	29 · 6
Control			24 · 4
Date mean	27 · 4	27 · 6	

S.E.D.: Treatment × Date = 3 · 74; Dates = 1 · 87; Treatments = 3 · 24.

(b) = mean shoot length (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	21 · 1	33 · 0	27 · 1
Daminozide	23 · 3	28 · 2	25 · 8
Tipped	29 · 3	27 · 0	28 · 1
Tied	25 · 1	27 · 2	26 · 2
Control			28 · 1
Date mean	24 · 7	28 · 9	

S.E.D.: Treatment × Date = 3 · 10; Dates = 2 · 45; Treatments = 2 · 68.

(c) = total shoot growth (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	543	802	672
Daminozide	572	797	684
Tipped	885	729	807
Tied	731	797	764
Control			679
Date mean	683	781	

S.E.D.: Treatment × Date = 120 · 8; Dates = 97 · 5; Treatments = 104 · 6.

(d) = shoot number 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	31 · 4	28 · 1	29 · 8
Daminozide	13 · 1*	10 · 4*	11 · 8*
Tipped	39 · 2	31 · 6	35 · 4
Tied	31 · 2	25 · 0	28 · 1
Control			30 · 9
Date mean	28 · 7	23 · 8	

S.E.D.: Treatment × Date = 6 · 42; Dates = 5 · 07; Treatments = 5 · 56.

Table 3.3.2.2 continued

(e) = mean shoot length (cm.) 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	34 · 4	36 · 1	35 · 2
Daminozide	25 · 1	13 · 5*	19 · 3*
Tipped	31 · 7	29 · 8	30 · 7
Tied	21 · 5	22 · 6	22 · 0
Control			30 · 2
Date mean	28 · 2	25 · 5	

S.E.D.: Treatment × Date = 4 · 90; Dates = 3 · 88; Treatments = 4 · 25.

(f) = total shoot growth (cm.) 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	1093	1038	1066
Daminozide	423	177*	306*
Tipped	1263	974	1119
Tied	760	589	674
Control			1044
Date mean	885	695	

S.E.D.: Treatment × Date = 268 · 1; Dates = 211 · 9; Treatments = 232 · 1.

(g) = number of spur buds produced in 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	7 · 94*	5 · 90	6 · 92
Daminozide	8 · 62*	5 · 64	7 · 13
Tipped	7 · 11	4 · 30	5 · 71
Tied	6 · 02	5 · 82	5 · 92
Control			4 · 87
Date mean	7 · 42*	5 · 42	

S.E.D.: Treatment × Date = 1 · 28; Dates = 1 · 63; Treatments = 1 · 12.

(h) = number of fruit set per 100 flower clusters

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	125 · 8	239 · 0*	182 · 4
Daminozide	123 · 0	200 · 4	161 · 7
Tipped	27 · 1	86 · 6	56 · 8
Tied	64 · 0	73 · 6	68 · 8
Control			110 · 2
Date mean	85 · 0	149 · 9	

S.E.D.: Treatment × Date = 40 · 54; Dates = 32 · 65; Treatments = 35 · 11.

Table 3.3.2.2 continued

(i) = initial set of spur fruit buds in 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	8 · 72*	7 · 97	8 · 33*
Daminozide	9 · 14	7 · 59	8 · 37*
Tipped	3 · 28	4 · 03	3 · 65
Tied	5 · 11	5 · 40	5 · 25
Control			4 · 26
Date mean	6 · 56	6 · 24	

S.E.D.: Treatment × Date = 1 · 63; Dates = 1 · 29; Treatments = 1 · 41.

(j) = percentage of initial set retained until final set assessment

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	51 · 6	41 · 4	46 · 5
Daminozide	61 · 5	75 · 3*	68 · 4*
Tipped	69 · 0*	62 · 6	65 · 8*
Tied	55 · 6	55 · 7	55 · 6
Control			34 · 8
Date mean	59 · 4	58 · 8	

S.E.D.: Treatment × Date = 14 · 26; Dates = 11 · 27; Treatments = 12 · 35.

(k) = fruit set of spur buds (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	5 · 28*	5 · 06	5 · 17*
Daminozide	6 · 92**	6 · 22*	6 · 57**
Tipped	2 · 01	2 · 89	2 · 45
Tied	3 · 61	3 · 79	3 · 70
Control			2 · 72
Date mean	4 · 45	4 · 49	

S.E.D.: Treatment × Date = 1 · 06; Dates = 6 · 84; Treatments = 6 · 92.

(l) = seed number of harvested fruit

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	7 · 23	7 · 21	7 · 22
Daminozide	7 · 18	7 · 22	7 · 20
Tipped	6 · 41	6 · 22	6 · 32
Tied	6 · 78	6 · 72	6 · 75
Control			6 · 86
Date mean	6 · 90	6 · 84	

S.E.D.: Treatment × Date = 0 · 36; Dates = 0 · 28; Treatments = 0 · 29.

plied in June and daminozide applied at either time significantly increased this (Table 3.3.2.2k). Fruit from trees where shoot tips had been removed at either time had the lowest number of seeds compared to all other treatments but differences were not significant (Table 3.3.2.2l).

3.3.2.3 Cox on MM106 rootstock

Unlike the Cox/M9 rootstock, the 1985 shoot growth of trees on MM106 rootstock was affected by some of the treatments imposed. Although shoot number was unaffected (Table 3.3.2.3a), individual shoot length was reduced by June application of daminozide (Table 3.3.2.3b). Paclobutrazol application at this time was also associated with a reduced mean shoot length very similar to that of the daminozide treated trees, but this was not significantly different to controls. However, the 30% reduction in individual shoot length on the daminozide treated trees contributed towards a 50% reduction in the total amount of shoot growth produced during 1985 (Table 3.3.2.3c).

In 1986, daminozide was again the only treatment to have any significant effects on shoot growth its affect being similar to that on Cox/M9. Both times of application were associated with a reduced number of shoots (Table 3.3.2.3d), the application in August also reducing individual shoot length (Table 3.3.2.3e). Control trees produced an average of 70 shoots each in 1986, compared to 29 and 13.6 on the trees treated with daminozide in June and August respectively. Because the August application also reduced individual shoot length by 50% compared to controls (Table 3.3.2.3e), total shoot growth of trees given this treatment was severely reduced to only $^{1}/_{10}$ of that on the control trees (Table 3.3.2.3f). Although daminozide in June had not significantly decreased individual shoot length, the reduction in shoot number associated with this treatment resulted in total shoot growth being only a half of that on control trees.

No treatment was associated with any changes in the number of fruit buds produced (Table 3.3.2.3g) but paclobutrazol applied in August and daminozide applied at either time all greatly increased the number of fruitlets set per cluster (Table 3.3.2.3h). This had the effect of significantly increasing the absolute number of fruitlets initially set - trees given either early or late daminozide applications setting 142 and 75 fruit per tree respectively compared to 24 per tree on controls (Table 3.3.2.3i).

Of the fruits initially set, no treatment significantly influenced the proportion which were retained until final set assessment (Table 3.3.2.3j). All trees lost a similar proportion of fruitlets during this time, and therefore the absolute number present at final set within each treatment were in the same proportions as they had been at initial set (Table 3.3.2.3k). The same treatments (i.e. daminozide at either time, paclobutrazol in June) were associated with significant increases in this. Unlike the other two cultivar/rootstock combinations studied, seed number

Tables 3.3.2.3a-l Components of shoot growth and fruit production in Cox trees (on MM106 rootstock) treated with Paclobutrazol, Daminozide or shoot tipping. Treatments were applied once, either in June or August 1985, shoot growth was measured in 1985 and 1986; components of fruit production were assessed in 1986. S.E.D. and significance levels refer to comparison with control (n=8). *, **, ***, indicate significance at the $P \leqslant 0 \cdot 05$, $0 \cdot 01$ or $0 \cdot 001$ levels respectively.

Tables are:-

(a) shoot number 1985
(b) mean shoot length 1985
(c) total shoot growth 1985
(d) shoot number 1986
(e) mean shoot length 1986
(f) total shoot growth 1986
(g) number of spur buds produced in 1986
(h) number of fruit set per 100 flower clusters
(i) initial set of spur fruit buds 1986
(j) percentage of initial set retained until final set assessment
(k) final set of spur fruit buds 1986
(l) seed number in harvested fruit

Table 3.3.2.3 (legend on facing page)

(a) = shoot number 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	45 · 2	52 · 6	48 · 9
Daminozide	33 · 2	41 · 1	37 · 2
Tipped	41 · 0	36 · 4	38 · 7
Control			48 · 3
Date mean	39 · 8	43 · 4	

S.E.D.: Treatment × Date = 6 · 62; Dates = 5 · 41; Treatments = 5 · 73.

(b) = mean shoot length (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	23 · 9	33 · 3	28 · 6
Daminozide	21 · 4*	31 · 9	26 · 7
Tipped	33 · 8	33 · 9	33 · 9
Control			30 · 7
Date mean	26 · 4	32 · 5	

S.E.D.: Treatment × Date = 2 · 80; Dates = 2 · 29; Treatments = 2 · 43.

(c) = total shoot growth (cm.) 1985

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	1071	1806	1438
Daminozide	726**	1319	1023
Tipped	1406	1216	1311
Control			1628
Date mean	1068*	1447	

S.E.D.: Treatment × Date = 241 · 2; Dates = 196 · 9; Treatments = 208 · 9.

(d) = shoot number 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	56 · 2	76 · 8	66 · 5
Daminozide	29 · 1**	13 · 6**	21 · 4**
Tipped	57 · 9	56 · 0	54 · 0
Control			70 · 8
Date mean	47 · 8*	46 · 8*	

S.E.D.: Treatment × Date = 10 · 73; Dates = 8 · 76; Treatments = 9 · 29.

Table 3.3.2.3 continued

(e) = mean shoot length 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	45 · 3	36 · 2	40 · 8
Daminozide	39 · 1	18 · 7**	28 · 9*
Tipped	46 · 1	34 · 4	40 · 3
Control			38 · 2
Date mean	43 · 5	29 · 7	

S.E.D. : Treatment × Date = 4 · 52; Dates = 3 · 70; Treatments = 3 · 93.

(f) = total shoot growth (cm.) 1986

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	2495	2727	2611
Daminozide	1224*	289**	757***
Tipped	2901	1897	2399
Control			2740
Date mean	2207	1638	

S.E.D. : Treatment × Date = 506 · 0; Dates = 413 · 1; Treatments = 438 · 2.

(g) = number of spur fruit buds produced in 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	12 · 36	11 · 13	11 · 74
Daminozide	11 · 96	10 · 19	11 · 08
Tipped	8 · 28	9 · 05	9 · 20
Control			10 · 89
Date mean	10 · 86	10 · 13	

S.E.D. : Treatment × Date = 1 · 24; Dates = 1 · 01; Treatments = 1 · 07.

(h) = number of fruit set per 100 flower clusters

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	34 · 5	110 · 7**	76 · 6*
Daminozide	107 · 8**	94 · 0**	100 · 9**
Tipped	27 · 6	33 · 3	30 · 5
Control			23 · 6
Date mean	56 · 6	79 · 3*	

S.E.D. : Treatment × Date = 24 · 37; Dates = 19 · 90; Treatments = 21 · 11.

Table 3.3.2.3 continued

(i) = initial set of spur fruit buds in 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	6 · 64	10 · 70***	8 · 67
Daminozide	11 · 94***	8 · 69**	10 · 31***
Tipped	2 · 35	4 · 36	3 · 35
Control			4 · 68
Date mean	6 · 97*	7 · 92**	

S.E.D.: Treatment × Date = 1 · 036; Dates = 0 · 846; Treatments = 0 · 897.

(j) = percentage of initial set retained until final set assessment

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	66 · 4	58 · 2	62 · 3
Daminozide	58 · 6	76 · 9	67 · 4
Tipped	61 · 2	90 · 4	76 · 1
Control			72 · 2
Date mean	61 · 9	75 · 3	

S.E.D.: Treatment × Date = 8 · 21; Dates = 6 · 70; Treatments = 7 · 11.

(k) = final set of spur buds in 1986 (under $\sqrt{}$ transformation)

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	5 · 23	8 · 05***	6 · 64*
Daminozide	8 · 49***	7 · 25**	7 · 89***
Tipped	1 · 67	4 · 11	2 · 89
Control			3 · 79
Date mean	5 · 13	6 · 47	

S.E.D.: Treatment × Date = 0 · 719; Dates = 0 · 587; Treatments = 0 · 623.

(l) = seed number in harvested fruit

Treatment	Time of application		Treatment
	June	August	mean.
Paclobutrazol	7 · 56*	6 · 06	6 · 81
Daminozide	7 · 16*	6 · 51	6 · 84*
Tipped	6 · 42	6 · 20	6 · 31
Control			6 · 03
Date mean	7 · 05	6 · 26	

S.E.D.: Treatment × Date = 0 · 34; Dates = 0 · 25; Treatments = 0 · 24.

in harvested fruit was influenced by treatment. Fruit from control trees had an average of 6.03 seeds each; those from trees treated with paclobutrazol or daminozide in June had 7.56 and 7.16 respectively (Table 3.3.2.3l).

3.3.2.4 Association between shoot growth and the components of cropping

This experiment showed that some of the regulatory treatments influenced some aspect of cropping and/or vegetative growth within each cultivar/rootstock combination. Various treatments had either reduced or increased shoot growth during either the year prior to, and/or the year of crop assessment. Simultaneously some very marked differences in the components of fruit production had taken place.

This provided an opportunity for examining in closer detail the degree of association (if any) which might exist between vegetative and reproductive growth. To this end the relationship between shoot growth components in both 1985 and 1986 and the components of fruit production in 1986 were analysed using regression analysis. By this means it was hoped to probe more deeply into whether any particular aspect of vegetative growth (i.e. shoot number, shoot length) either in the year prior to, or during cropping was strongly associated with any component of fruit production.

Since large cultivar differences in response to different treatments had been observed, the degree of association between all vegetative and reproductive measurements was assessed within both. However, because within Cox no major rootstock/treatment interactions had been seen, data from trees on both rootstocks were combined to give a greater number of trees to compare.

Regression coefficients, standard errors and significance of the regressions are presented in Tables 3.3.2.4 and - 3.3.2.5. Unless stated otherwise, all relationships mentioned in the text are significant at $P \leqslant 0.05$.

Within both cultivar/rootstock situations, regression analysis indicated that various components of fruit production were related to various aspects of shoot growth.

Within both Cox and Bramley the total amount of shoot growth produced during 1985 was related to the number of fruit buds produced and the number of fruits initially set (Tables 3.3.2.4. and 3.3.2.5). However, the expression of this was different in the two varieties. Within Bramley, the number of flowers produced and the number of fruits which initially set tended to increase as total shoot growth decreased (Table 3.3.2.4), whereas within the Cox, increased levels of shoot production in 1985 were associated with increasing numbers of both fruit bud produced and fruitlets set (Table 3.3.2.5).

This pattern was repeated in the relationships between the total shoot growth produced in 1986 and the fruit production components; in Bramley increased shoot production was associated with decreased flower and fruit production but within Cox, although fewer relationships

Tables 3.3.2.4a-f Regression co-efficients, standard errors and significance of the regression of components of shoot growth (in 1985 & 1986) on components of fruit production (in 1986 in Bramley on M26 rootstock. *,**,***, indicate regressions significantly different from zero at $P \leqslant 0 \cdot 05, 0 \cdot 01, 0 \cdot 001$ respectively.

Tables are:-

(a) total shoot growth 1985
(b) shoot number 1985
(c) mean shoot length 1985
(d) total shoot growth 1986
(e) shoot number 1986
(f) mean shoot length 1986

Table 3.3.2.4 (legend on facing page)

(a) = total shoot growth 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)			ns	
initial set of spurs ($\sqrt{}$)	−0 · 3221	0 · 0749	***	16 · 7
final set of spurs ($\sqrt{}$)	−0 · 2036	0 · 0511	***	14 · 3
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)	−0 · 2403	0 · 0931	*	6 · 0
final set of axilliaries			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

(b) = shoot number 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	−0 · 1152	0 · 0316	***	12 · 2
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)			ns	
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)			ns	
final set of axilliaries			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

(c) = mean shoot length 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	−0 · 052	0 · 011	***	18 · 5
initial set of spurs ($\sqrt{}$)	−0 · 067	0 · 012	***	25 · 6
final set of spurs ($\sqrt{}$)	−0 · 039	0 · 009	***	18 · 6
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)	−0 · 062	0 · 015	***	15 · 2
final set of axilliaries			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

Table 3.3.2.4 continued

(d) = total shoot growth 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)			ns	
initial set of spurs ($\sqrt{}$)	−0 · 084	0 · 012	***	36 · 2
final set of spurs ($\sqrt{}$)	−0 · 0661	0 · 0071	***	49 · 2
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)	−0 · 0914	0 · 0144	***	30 · 7
final set of axilliaries			ns	
% of buds which produced a fruit	−1 · 152	0 · 532	*	4.6
% initial set remaining until final set			ns	

(e) = shoot number 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	−0 · 01184	0 · 00058	*	3 · 4
initial set of spurs ($\sqrt{}$)	−0 · 03499	0 · 00543	***	31 · 5
final set of spurs ($\sqrt{}$)	−0 · 0286	0 · 0033	***	46 · 0
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)	−0 · 0360	0 · 0068	***	23 · 6
final set of axilliaries			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

(f) = mean shoot length 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	−0 · 0190	0 · 00935	*	3 · 4
initial set of spurs ($\sqrt{}$)	−0 · 0587	0 · 00858	***	34 · 4
final set of spurs ($\sqrt{}$)	−0 · 0427	0 · 0055	***	39 · 9
number of axilliary buds ($\sqrt{}$)			ns	
initial set of axilliaries ($\sqrt{}$)	−0 · 0701	0 · 0099	***	35 · 4
final set of axilliaries			ns	
% of buds which produced a fruit	−1 · 016	0 · 378	**	6 · 6
% initial set remaining until final set			ns	

Tables 3.3.2.5a-f Regression co-efficients, standard errors and significance of the regression of components of shoot growth (in 1985 & 1986) on components of fruit production (in 1986) in Cox on M9 & MM106 rootstocks. *,**,***, indicate regressions significantly different from zero at $P \leqslant 0 \cdot 05, 0 \cdot 01, 0 \cdot 001$ respectively.

Tables are:-

(a) total shoot growth 1985
(b) shoot number 1985
(c) mean shoot length 1985
(d) total shoot growth 1986
(e) shoot number 1986
(f) mean shoot length 1986

(a) = total shoot growth 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	0 · 3211	0 · 0504	***	24 · 2
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)	0 · 1346	0 · 0457	**	5 · 8
% of buds which produced a fruit	3 · 16	1 · 42	*	3 · 2
% initial set remaining until final set			ns	

(b) = shoot number 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	0 · 1494	0 · 0181	***	34 · 9
initial set of spurs ($\sqrt{}$)	0 · 0656	0 · 0253	*	4 · 3
final set of spurs ($\sqrt{}$)	0 · 0641	0 · 0174	***	9 · 1
% of buds which produced a fruit	−1 · 237	0 · 549	*	3 · 2
% initial set remaining until final set			ns	

Table 3.3.2.5 continued

(c) = mean shoot length 1985

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)			ns	
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

(d) = total shoot growth 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	0 · 1271	0 · 0236	***	19 · 2
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)			ns	
% of buds which produced a fruit	−1 · 987	0 · 644	**	6 · 8
% initial set remaining until final set			ns	

(e) = shoot number 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	0 · 0557	0 · 0111	***	16 · 9
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)			ns	
% of buds which produced a fruit	−1 · 237	0 · 549	*	3 · 2
% initial set remaining until final set			ns	

(f) = mean shoot length 1986

	co-efficient of variations	standard error	significance level	% variations accounted for
number of spur buds ($\sqrt{}$)	0 · 0882	0 · 0232	***	10 · 4
initial set of spurs ($\sqrt{}$)			ns	
final set of spurs ($\sqrt{}$)			ns	
% of buds which produced a fruit			ns	
% initial set remaining until final set			ns	

between these characters were found, the number of flowers increased with shoot growth, but the percentage of these which subsequently set decreased with increasing growth. Within Bramley, fruit production was very significantly and inversely related to all aspects of shoot growth in 1986. As shoot number, individual shoot length and consequently, total shoot growth increases, flower production, fruit set and fruitlet retention decreased.

3.3.3 Experiment 3:

Effects of branch angle and tree age on shoot growth and flower initiation in young trees.

After the first growing season it was apparent that the various treatments had produced trees with quite different shapes (Plate 3.3.3.1).

On average, the trees on M9 had 11.4 shoots with a mean length of 36.2 cm. combining to give a total shoot growth of 410.7cm. (Table 3.3.3.1). Trees on MM106 had slightly more growth, with an average of 16.5 shoots per tree, each with a mean length of 30.4 cm. giving a total shoot growth of 515.2 cm. (Table 3.3.3.2). Branch orientation did not significantly affect any of these components of shoot growth within trees on either rootstock.

Similarly the number of fruit buds initiated in 1985 and the number of these which set fruit were also unaffected by branch orientation. Trees on M9 rootstock had an average of 27 fruit buds per tree from which only 4 fruit were initially set and only 2 remained at finally set assessment (Table 3.3.3.1). Within the trees on MM106 rootstock, an average of 48.2 fruit buds were produced per tree of which 7 set initially and 3.5 remained at final set (Table 3.3.3.2).

This does not agree with the reports suggesting that horizontally orientated branches grow less and produce more flowers and fruit than do vertical ones.

However, it was seen that although shoots had been trained to a horizontal position in April, shoot tips had subsequently grown in an upright direction (Plate 3.3.3.1a). This treatment was therefore not assessing the effects of having horizontal shoot tips. Consequently, in the following year (1985) although all orientations were 'reset', the group of trees designated to have horizontal branches were subdivided. Within each block, half of these trees had a repeat of the treatment imposed in 1984 - that is, branches were tied to horizontal positions at the start of the season but shoots were then allowed to grow in an upwards direction. The remaining trees also had branches tied down at the start of the season but they were then monitored at weekly intervals and weighted pegs were used to maintain the horizontal orientation of the shoot tips.

Unlike during the first growing season, shoot growth within trees on both rootstocks in 1985 was affected by branch orientation. Although the number of shoots produced by trees on M9 rootstock were not affected by treatments, the individual shoot length and the total amount of shoot production was. Of trees on M9 rootstock, where branches had been maintained in a horizontal orientation throughout the season, individual shoot length was about $^{2}/_{3}$ of that

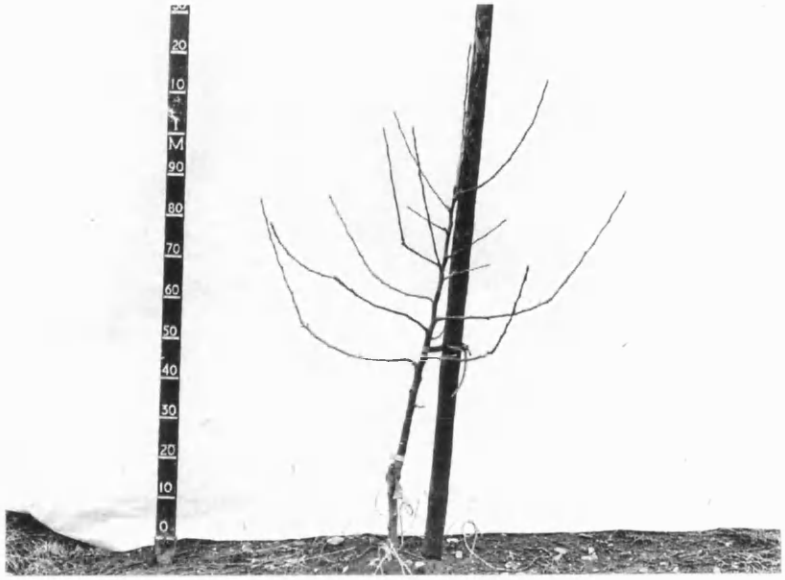
Plate 3.3.3.1 One-year-old Cox/M9 where branches had been trained to

(a) a horizontal position,

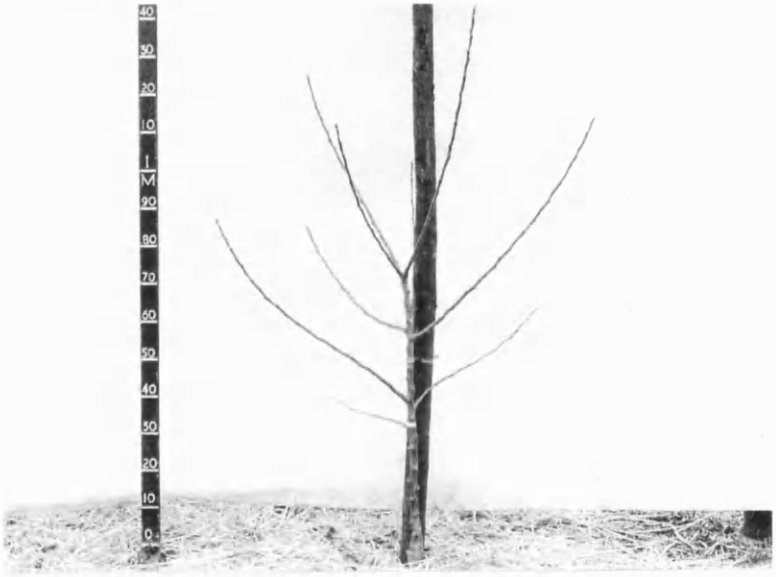
(b) 45°

(c) a vertical position at the start of the season.

a)


20
10
1
M
90
80
70
60
50
40
30
20
10
0

b)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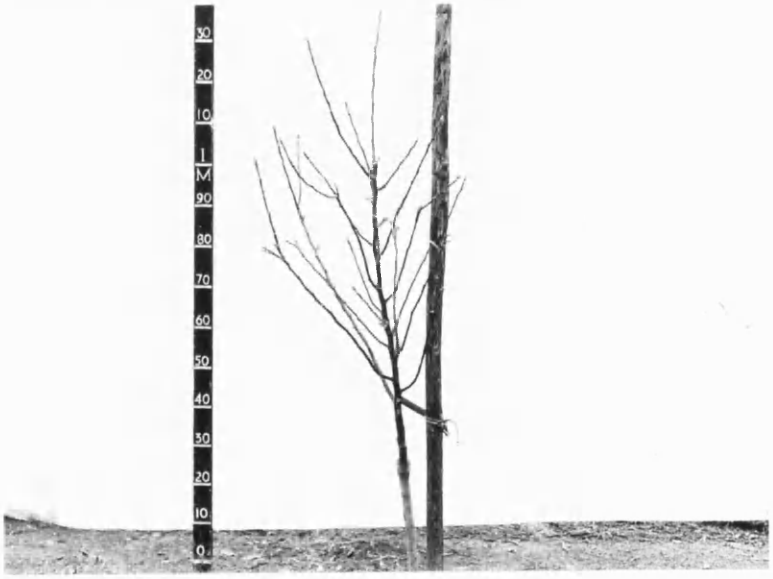
30
20
10
1
M
90
80
70
60
50
40
30
20
10
0

c)


30
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50
40
30
20
10
0

Tables 3.3.3.1a + b Shoot growth (a) and fruit production (b) of Cox trees on M9 rootstock. At the start of the 1984 season branches were trained to a horizontal, vertical or intermediate (45°) position - direction of shoot growth was unrestricted. In 1985 treatments were reapplied to the same trees except that any original shoot-tip orientation was maintained throughout the season. On one group of trees where branches were trained horizontal in 1985, shoot tips were once again permitted to grow in an upright direction. Values bearing the same letter do not differ significantly at $P \leqslant 0 \cdot 05$.

(a) = shoot growth

		horizontal/ tips upright	horizontal/ tips horiz.	45°	vertical	S.E.D.
year 1	total shoot growth (m.)	4 · 06a	-	3 · 89a	4 · 30a	0 · 356
(1984)	shoot number	12 · 5a	-	10 · 9a	11 · 1a	1 · 3
	mean shoot length (cm.)	32 · 5a	-	35 · 3a	38 · 9a	3 · 2
year 2	total shoot growth (m.)	8 · 84ab	6 · 11a	11 · 45b	8 · 95ab	1 · 36
(1985)	shoot number	28 · 9a	28 · 8a	39 · 7a	29 · 5a	5 · 88
	mean shoot length (cm.)	31 · 1b	21 · 6a	29 · 3b	30 · 7b	3 · 017
year 3	total shoot growth (m.)	15 · 42ab	10 · 97a	20 · 93b	15 · 57ab	3 · 24
(1986)	shoot number	38 · 0ab	31 · 5a	46 · 9b	43 · 1ab	6 · 6
	mean shoot length (cm.)	34 · 9a	34 · 0a	45 · 1b	35 · 8a	4 · 1

(b) = fruit production

		horizontal/ tips upright	horizontal/ tips horiz.	45°	vertical	S.E.D.
year 2	number of fruit buds	27 · 8a	-	24 · 5a	28 · 5a	3 · 6
(1985)	number of fruits initially set	4 · 3a	-	4 · 0a	4 · 7a	1 · 9
	number of fruits finally set	1 · 9ab	-	2 · 0a	2 · 1a	1 · 6
year 3	number of fruit buds	66 · 2ab	81 · 8b	60 · 7a	51 · 1a	9 · 7
(1986)	number of fruits initially set	108 · 3b	132 · 4b	67 · 3a	53 · 8a	16 · 1
	number of fruits finally set	53 · 3c	60 · 2c	38 · 9b	22 · 2a	11 · 2

Tables 3.3.3.2a + b Shoot growth (a) and fruit production (b) of Cox trees on MM106 rootstock. At the start of the 1984 season branches were trained to a horizontal, vertical or intermediate (45°) position - direction of shoot growth was unrestricted. In 1985 treatments were reapplied to the same trees except that any original shoot-tip orientation was maintained throughout the season. On one group of trees where branches were trained horizontal in 1985, shoot tips were once again permitted to grow in an upright direction. Values bearing the same letter do not differ significantly at $P \leqslant 0 \cdot 05$.

(a) = shoot growth

		horizontal/ tips upright	horizontal/ tips horiz.	45°	vertical	S.E.D.
year 1	total shoot growth (m.)	5 · 07a	-	4 · 64a	5 · 23a	0 · 55
(1984)	shoot number	17 · 9a	-	15 · 1a	16 · 9a	1 · 92
	mean shoot length (cm.)	29 · 1a	-	31 · 0a	30 · 8a	3 · 14
year 2	total shoot growth (m.)	12 · 92a	10 · 72a	13 · 91b	11 · 59a	2 · 38
(1985)	shoot number	46 · 1a	48 · 7a	42 · 1a	34 · 5a	6 · 7
	mean shoot length (cm.)	29 · 3ab	22 · 0a	33 · 7b	32 · 6b	3 · 0
year 3	total shoot growth (m.)	15 · 72ab	13 · 43a	21 · 71b	18 · 15ab	3 · 31
(1986)	shoot number	58 · 7a	53 · 4a	49 · 8a	48 · 8a	10 · 6
	mean shoot length (cm.)	27 · 9ab	25 · 7a	42 · 8c	36 · 7bc	4 · 5

(b) = fruit production

		horizontal/ tips upright	horizontal/ tips horiz.	45°	vertical	S.E.D.
year 2	number of fruit buds	48 · 2a	-	46 · 2a	50 · 2a	6 · 33
(1985)	number of fruits initially set	6 · 8a	-	7 · 2a	6 · 5a	1 · 9
	number of fruits finally set	3 · 1a	-	3 · 8a	3 · 5a	1 · 3
year 3	number of fruit buds	104 · 6ab	124 · 3b	66 · 6a	64 · 0a	21 · 7
(1986)	number of fruits initially set	81 · 1b	87 · 3b	37 · 8a	21 · 0a	18 · 9
	number of fruits finally set	41 · 3c	47 · 1c	29 · 1b	15 · 0a	8 · 9

grown on trees given any other treatment and total shoot growth only about half of that on trees where branches had been at 45°. Where branches had either been trained horizontally and then allowed to grow or had been trained to a vertical position, trees had produced similar quantities of shoot growth and were intermediate between the other two treatments (Table 3.3.3.1).

Similar to the situation within the trees on M9 rootstock, within those on MM106 rootstock, individual shoot length of trees where shoots had been maintained in horizontal position was reduced compared to all other treatments. This did not result in the total amount of shoot growth produced being significantly reduced (Table 3.3.3.2).

Fruit bud number, initial and final fruit set in 1986 all showed differences associated with the different orientation treatments, the overall pattern being consistent between the two rootstocks. All three counts were consistently highest within the trees where branches had been maintained in a horizontal orientation. Within those on M9 rootstock, 81 fruit buds were formed on these trees compared to 51 on trees with vertical branches. This was followed by a greater number of fruit being set per cluster on these trees, and the two factors combined to give them more than double the number of fruitlets initially set compared to trees where branches had been either vertical or at 45° (Table 3.3.3.1). No differences were seen in the proportion of fruitlets which were retained until final set assessment, and as such, trees where branches had been maintained in a horizontal position had significantly more fruit present at this time than did trees with vertical branches. Trees with branches at 45° or trained horizontally but allowed to grow were intermediate in this.

Within the trees on MM106 rootstock the situation was very similar. Trees with horizontal branches produced more fruit bud than did those with branches either vertical or at 45° but this was only significant within the trees where shoot tips had been maintained in a horizontal orientation (Table 3.3.3.2). Because there were no detectable differences in the proportion of these buds which produced fruitlets or fruitlets which were retained, there were significantly more fruits initially and finally set on trees with horizontally maintained shoots than there were on trees with vertical branches.

3.3.4 Experiment 4:

Effects of altering branch angle, either during or after seasonal growth, on fruitlet retention, shoot growth, fruit-bud formation and fruit set.

During the time of fruitlet collection there were effectively only three branch orientation treatments; i.e. trees with upright branches and those which had had branches tied into a horizontal position on either April 23rd or June 4th. Trees where branches had been tied down in April had a reduced number of fruit initially set compared to the other treatments (Table 3.3.4.1). Following this, no treatment associated differences in either the number of fruit dropped, nor

Table 3.3.4.1. Fruit set and retention on young Discovery trees during the season of branch angle alteration. Branch orientation was changed from predominantly upright to a horizontal position in either April, May, July or October 1984. * indicates significance at the $P \leqslant 0 \cdot 05$ level.

	time of altering branch orientation					
	April	May	July	October	S.E.D.	Probability
number of fruit buds	48 · 9	46 · 7	46 · 9	47 · 3	2 · 43	ns
number of fruit initially set	92 · 1	110 · 1	115 · 2	117 · 0	7 · 41	*
number of fruit final set	6 · 5	11 · 5	16 · 5	18 · 5	6 · 06	ns
% loss (initial set → harvest)	94 · 6	92 · 5	87 · 6	89 · 8	4 · 6	ns
total fruit drop/100 flower clusters	175	211	210	208	24 · 8	ns

their individual or combined weights, either within each collection period or over the collection time as a whole were apparent (Tables 3.3.4.2a - c).

When the proportion of fruit drop which occurred during each individual period was calculated, no differences between treatments were seen, approximately equal numbers of fruit being shed during each time interval by the trees within each treatment (Table 3.3.4.2d).

The number of fruit present at final harvest was very low compared to both the number of flower buds and the number of fruit initially set (Table 3.3.4.1). Initial fruit set was good, averaging approximately two fruit per cluster, but approximately 90% of these were shed before maturity.

Thus it would appear that in these young Discovery trees, fruitlet retention may be a more important cause of poor cropping than is poor fruit set. Altering branch angle prior to either flowering or June drop did not appear to have any effect on this.

Harvested fruit quality was good, the majority of fruit being either class 1 or extra class, and was not influenced significantly by altering branch angle during the season (Table 3.3.4.3).

Shoot growth during the season was unaffected by branch angle (Table 3.3.4.4); no differences in total shoot growth, mean shoot length or the number of shoots per tree being detected between treatments.

The number of fruit buds formed the year following alteration of branch angle was influenced by the time of treatment. Generally, the earlier in the year that branches had been tied into a horizontal position, the greater the number of fruit buds formed (Table 3.3.4.4). Trees where branches had been trained to a horizontal position at the first tying date (April 23rd) produced an average of 296 buds per tree, significantly more than those where branches had been trained at the end of the season or had remained vertical throughout.

The number of fruit initially set was also affected by the treatments in the same manner, trees in which branches had been tied into a horizontal position on April 23rd set more fruit than did those where branches had been tied at the end of the season or had remained vertical throughout. However, no differences were observed in the proportion of flowers which set fruit, the number retained until harvest, nor their individual weight (Table 3.3.4.4).

3.4 Discussion

Results from the various experiments reported in this chapter demonstrate that within young trees it is indeed possible to increase both the number of flower clusters formed and the proportion of these which set fruit. This was achieved by both cultural and chemical means. However, this increase in fruit number was often associated with an undesirable decrease in vegetative growth.

When the problem of how to encourage fruit production in young trees was approached from a different angle, and they were sprayed with gibberellin in an attempt to increase their

Tables 3.3.4.2a-d Pattern of fruitlet drop occurring during 'June drop' in Discovery trees where branches remained upright or had been tied to a horizontal position approximately 3 or 8 weeks earlier. Data presented are (a) number of fruitlets dropped, (b) weight of fruitlets dropped, (c) total weight of dropped fruit and (d) percentage of total dropped fruit collected at each harvest date.

(a) = number of fruitlets dropped

	time of altering branch orientation				
date of fruitlet collection	April 23rd	June 4th	remained upright	S.E.D. ( )*	probability
25th June	31 · 5	40 · 5	35 · 9	8 · 53 (7 · 38)	ns
30th June	20 · 6	23 · 6	23 · 7	4 · 16 (3 · 60)	ns
3rd July	17 · 6	18 · 6	21 · 9	4 · 28 (3 · 71)	ns
5th July	5 · 0	3 · 9	6 · 3	1 · 52 (1 · 32)	ns
10th July	10 · 7	12 · 0	10 · 6	1 · 82 (1 · 56)	ns
total number dropped	85 · 5	98 · 6	98 · 5	14 · 42 (12.49)	ns
number of trees used (reps)	11	11	22		

* = S.E.D. when comparing min - max replicates.

(b) = mean weight (g) of fruitlets dropped

	time of altering branch orientation				
date of fruitlet collection	April 23rd	June 4th	remained upright	S.E.D. ( )*	probability
25th June	1 · 49	1 · 66	1 · 60	0 · 141 (0 · 122)	ns
30th June	4 · 86	4 · 87	4 · 81	0 · 282 (0 · 245)	ns
3rd July	5 · 53	7 · 18	5 · 22	1 · 087 (0 · 942)	ns
5th July	6 · 24	7 · 39	7 · 29	0 · 743 (0 · 644)	ns
10th July	6 · 95	7 · 45	7 · 47	0 · 454 (0 · 393)	ns
mean of all fruit dropped	4 · 37	4 · 79	4 · 51	0 · 313 (0 · 271)	ns

* = S.E.D. when comparing min - max replicates.

Tables 3.3.4.2. continued

(c) = total weight of fruitlets dropped

date of fruitlet collection	time of altering branch orientation: April 23rd	June 4th	remained upright	S.E.D. ( )*	probability
25th June	1 · 49	1 · 66	1 · 60	0 · 141 (0 · 122)	ns
30th June	4 · 86	4 · 87	4 · 81	0 · 282 (0 · 245)	ns
3rd July	5 · 53	7 · 18	5 · 22	1 · 087 (0 · 942)	ns
5th July	6 · 24	7 · 39	7 · 29	0 · 743 (0 · 644)	ns
10th July	6 · 95	7 · 45	7 · 47	0 · 454 (0 · 393)	ns
mean of all fruit dropped	4 · 37	4 · 79	4 · 51	0 · 313 (0 · 271)	ns

* = S.E.D. when comparing min - max replicates.

(d) = percentage of total fruit drop collected on each harvest date

date of fruitlet collection	time of altering branch orientation: April 23rd	June 4th	remained upright	S.E.D. ( )*	probability
25th June	34 · 0	33 · 0	30 · 5	4 · 53 (3 · 95)	ns
30th June	24 · 5	23 · 0	22 · 6	4 · 57 (3 · 96)	ns
3rd July	20 · 2	20 · 0	19 · 8	3 · 43 (2 · 97)	ns
5th July	4 · 9	4 · 0	5 · 8	1 · 66 (1 · 44)	ns
10th July	11 · 3	12 · 4	8 · 8	1 · 74 (1 · 51)	ns

* = S.E.D. when comparing min - max replicates.

Table 3.3.4.3 Quality of harvested fruit as affected by branch angle alteration during the season

fruit quality	time of altering branch orientation 23rd April	4th June	30th July	1st October	S.E.D.	probability
extra class	53 · 3	43 · 3	37 · 9	51 · 7	7 · 83	ns
class 1	31 · 4	36 · 2	40 · 0	39 · 7	4 · 54	ns
class 2 + 3	12 · 3	16 · 5	19 · 6	10 · 8	4 · 21	ns
unmarketable	3 · 03	4 · 03	2 · 49	1 · 71	1 · 224	ns

Table 3.3.4.4. Shoot growth (1984) and flower and fruit production (1985) of young Discovery trees in which branches had either been changed from a predominantly upright to a horizontal position at one of four times during 1984 or had remained upright throughout 1984 and 1985. *, ** indicate significance at $P \leqslant 0 \cdot 05$ and $0 \cdot 01$ respectively.

	time of altering branch orientation 23 April	4 June	30 July	1 Oct	unchanged	S.E.D.	probability
total shoot growth (m) during 1984	3 · 77	3 · 60	3 · 89	3 · 91	3 · 87	0 · 349	ns
shoot number (1984)	111 · 4	118 · 6	114 · 6	108 · 8	116 · 4	7 · 48	ns
number of fruit buds (1985)	296 · 0	275 · 2	254 · 4	239 · 9	235 · 6	13 · 94	**
number of fruits initially set (1985)	262 · 5	221 · 3	224 · 0	199 · 1	202 · 3	29 · 91	*
number of fruits set per 100 flower cluster	88 · 0	80 · 4	96 · 1	82 · 3	84 · 6	8 · 31	ns
number of fruits finally set	79 · 1	93 · 5	99 · 8	96 · 0	88 · 3	13 · 07	ns

vegetative, rather than reproductive growth, results were disappointing in that gibberellin appeared to have little effect.

Because in fruit trees flower bud formation and shoot growth are closely interrelated, any attempt to affect one of them without affecting the others has hardly any chance of success. Thus these parameters shall be discussed together in relation the various treatment effects upon them.

3.4.1 Influence of chemical treatments on flower initiation and shoot growth

The small effect of gibberellin en shoot growth and flower initiation was unexpected. There are many references in the literature reporting gibberellin application to increase shoot growth (Greenhalgh and Edgerton 1967, Jackson and Street 1972) and to decrease the number of flowers produced (Dennis and Edgerton 1966, Marino and Greene 1981). Even where shoot growth is not significantly increased, some suppression of flower number is often seen (Tromp 1982, Marino and Greene 1981). In the experiments described here, the results were very different, in one case, treatment with 50 ppm GA_3 in May to Cox/M9 decreased shoot growth and increased the number of spur buds formed. No other reports of this effect are known. It was surprising that it was not the highest gibberellin concentration which exerted an effect but Luckwill (1970) also found that higher concentrations of gibberellin were not always associated with the greatest effects. However, although in no case were spur fruit buds adversely affected by gibberellin application, axillary fruit bud formation was. All June gibberellin applications severely reduced the number of axillary fruit buds formed on Cox/M9 and 500 ppm in May reduced their number on Cox/MM106. None of these reductions in fruit bud formation were associated with any significant increase in shoot growth. Tromp (1982) also found fruit bud number but not shoot growth to be affected by gibberellin application. It is interesting that he also found later applications (full bloom plus 4 weeks) to be more effective at decreasing flower number on current years wood than were earlier ones (full bloom). He also noted that the early sprays more effectively reduced the number of fruit buds on older wood, and concluded from this that gibberellin affected the early, vegetative stage of bud production. If floral bud development begins earlier on older, compared to younger wood (Zeller 1960) then the late sprays would be ineffective at reducing the number of buds on the former because buds would have already begun development. Within the experiments described in this chapter, no other treatment was associated with a decrease in the number of fruit buds even although removal of shoot tips in June resulted in increased shoot growth and therefore might therefore have been expected to do so (Elfving and Forshey 1976a). On the contrary, several treatments increased the number of fruit buds.

Treatment with paclobutrazol, daminozide or branch bending in June or paclobutrazol in August all increased fruit bud number in Bramley as did paclobutrazol or daminozide appli-

cation in June to Cox/M9. Of these only the June applications of paclobutrazol or daminozide to Bramley was associated with any significant reduction in shoot growth relative to controls. There are many conflicting reports in the literature regarding the relationship between shoot growth and flower initiation. Although the antagonism between them is widely accepted (Lang 1961, Forshey 1978, Dennis 1979), whether or not the two are irreversibly linked is less so. There are numerous reports of experimental treatments which cause a decrease in shoot growth also causing an increase in fruit bud number the following year (Wareing and Nasr 1958, Veinbrants 1972, Williams 1972). Similarly treatments which enhance either the duration or vigour of shoot growth are often followed by decreased fruit bud production (Batjer and Westwood 1963, Greenhalgh and Edgerton 1967, Hansen 1980). However, there are also reports where shoot growth or flower number are altered with no apparent effect on the other (Volz and Knight 1986, Tromp 1987).

One theory put forward to explain the antagonism between shoot growth and flowering is that whilst a shoot tip is actively growing, the shoot apex produces a growth substance inhibiting flower formation (Wareing and Nasr 1961). Because developing young leaves are a rich source of gibberellins (Kato and Ito 1962, Tromp and Wertheim 1980) and the inhibitory effects of gibberellin on flowering have been demonstrated in many experiments (Dennis and Edgerton 1966, Marino and Greene 1981, Tromp 1982), it seems likely that this substance may well be a gibberellin as suggested by Luckwill (1970). Only when growth slows or terminates, and gibberellin levels decrease can buds below the shoot tip increase their meristematic activity, and perhaps, more successfully compete for nutrients. Other evidence suggesting that gibberellin might be the inhibitor of flowering is the fact that both TIBA (an inhibitor of IAA and GA transport) and paclobutrazol, an anti-gibberellin (Hedden and Graebe 1985), stimulate flower initiation in young trees (Luckwill 1970, Tukey 1983, Tromp 1987).

The main prerequisite for flower bud formation is a critical number of nodes (Luckwill 1974, Abbott 1977), and the length of time between each node being formed (the plastochron) is critical in determining whether or not a bud will have reached sufficient complexity to become floral prior to dormancy (Fulford 1966). If a bud has about 6 nodes at the start of the season and a plastochron of seven days (both of which are realistic possibilities) then the bud should reach sufficient complexity for floral initiation to occur in early August (Tromp and Wertheim 1980). If the plastochron is much shorter than this the bud may immediately commence growth as a leafy shoot. Conversely if the plastochron is longer, then the bud may never attain the required number of nodes for floral initiation and therefore remain vegetative (Luckwill 1974). In some of the references to the antagonism between growth and flowering it has been suggested that treatments which reduce shoot activity also reduce apical dominance and allow a greater level of meristmatic activity within subtending buds (Wareing and Nasr 1961, Luckwill 1968). Luckwill (1974) suggested that growth regulators may affect flowering by shorten-

ing the plastochron such that by the end of the growing season more buds will have reached the critical number of nodes and fruit bud number the next year would be increased. Luckwill and Silva (1979), however, found that neither daminozide nor GA affected the rate of node production although daminozide did increase the proportion of buds which initiated flowers.

It is still possible however, that conditions which favour growth will maintain a slow development of potential fruit buds and it therefore seems possible that any treatment which either slows vegetative growth, or encourages it to stop early could encourage faster bud development, and possibly an increased bud number the following year.

It is also possible that in addition to having an indirect effect through growth suppression, treatments may also directly affect flower initiation and many people have come to this conclusion after consideration of experimental results.

Firstly, Batjer *et al.* (1964) demonstrated that by spraying only the lower third of a tree with daminozide, shoot growth was only decreased in the sprayed part but flower production was enhanced over the whole tree. They suggested that perhaps the regulator had been translocated to the roots, where it influenced some hormone production which subsequently increased flower initiation throughout the tree: however Williams (1973) repeated the experiment and found that daminozide was readily translocated to the upper part of the tree where it then affected shoot growth. It is possible that in Batjers' situation the growth regulator translocated to the upper part of the tree may have arrived in sufficient concentration to influence flower formation but insufficient to affect shoot growth.

Also, again using daminozide, Luckwill (1970) found that the optimum concentrations for either reducing shoot growth or enhancing flower formation were different, and concluded therefore that the chemical was acting directly upon both phenomena.

Similar situations have been seen whereby daminozide has strongly inhibited vegetative growth of apple seedlings but with no concurrent increase in flowering, and in the same experiment, has increased the flowering of pear seedlings even though there was no vegetative response (Dennis 1968).

Much investigation into this question has been done by Tromp (1968, 1970, 1972, 1987) who, using several different techniques has altered the level of flower initiation without apparently affecting shoot growth (Tromp 1972). He also showed that the number of flower buds produced could be increased by imposing certain treatments after cessation of shoot growth; a situation where alteration of growth could have had nothing to do with the observed increase in flowering (Tromp 1970, 1972).

Superficially, the results obtained in the experiments described here are open to a similar interpretation: that shoot growth does not have to be reduced in order for increased flower initiation to take place. However closer examination of the results does reveal some relationships between growth and flower production. Although within Bramley paclobutrazol applica-

tion in August was associated with an increase in bud number and no significant decrease in shoot growth, the amount of growth on these trees was, on average, less than on trees given any other treatment in August (Table 3.3.2.1c). Similarly within Cox/M9, although not significantly different from controls, trees treated with paclobutrazol or daminozide in June had the least amount of shoot growth during 1985 compared to trees given any other treatment. Although Williams (1973) pointed out that in order to be active in flower initiation, a chemical application or cultural practice must alter the general growth processes during the flowering period, he did indicate that this need only be temporary. Thus it is possible that the final amount of shoot growth does not have to be significantly reduced in order for flower initiation to occur, if instead it is interrupted or reduced at a critical time. That this can indeed be the case was shown by Quinlan and Preston (1973); where the temporary halting of shoot growth by chemical pinching agents greatly stimulated axillary bud development.

However, from the regression analyses it was very clear that (within Bramley at least) high levels of fruit bud production were found on trees with low levels of vegetative growth. But even with this data, it is still impossible to determine whether the two phenomena are connected directly to one another or just individual expressions of the way in which the treatments have exerted an effect on both of them.

If the two phenomena really are separate, then in what ways might the treatments be exerting their effect?

Since it is widely accepted that an intricate balance of endogenous hormones must prevail before flower initiation can occur, a possible direct effect of exogenously applied chemicals is to interfere with the production and utilisation of the endogenous growth regulators.

Paclobutrazol is known to be an anti-gibberellin, inhibiting a stage in its synthesis (Dalziel and Lawrence 1984, Hedden and Graebe 1985) but the mode of action of daminozide is more uncertain. It is thought to be antagonistic to GA (Saure 1978) and the growth inhibition caused by it can be counteracted with high concentrations of potassium gibberelate (Edgerton and Hoffman 1965). Williams and Stahley (1970) found it to interfere with auxin production and Hoad and Monselise (1976) found that within 2 days of spraying M26 rootstocks with daminozide, ABA increased within the tissues of the shoot tip and 5 days later, a decrease in gibberellin-like substances was observed. In a similar experiment but on fruiting trees, only a slight, and non significant decrease in the amount of gibberellin like substances diffusing from shoot tips was seen after spraying with daminozide two weeks after full bloom (Ramirez and Hoad 1978). As Ramirez and Hoad (1978) pointed out, although it is suggested that GA application decreases flower initiation because it stimulates growth (Jackson and Sweet 1972), work using growth regulators has shown situations where a growth retardant can affect both growth and flower initiation but have no observable effect on GA content of tissues.

Another way in which daminozide might affect flower initiation is through phosphorus metabolism; plants treated with phosphorus plus daminozide producing more buds than did those treated with either substance alone (Williams and Thompson 1979).

But Luckwill (1970) suggested that other than affecting flowering by interfering with extension growth, growth retardants may also exert an influence by increasing the concentration of cytokinins within the xylem sap as reported to occur within the sap of *Vitus vinifera* (Skene 1968) and that this increase in cytokinin concentration may stimulate activity within the buds. Such a hypothesis would explain why growth retardants can sometimes increase floral initiation even in situations where there is no reduction in shoot growth (Veinbrants 1972, Williams 1973).

It has also been shown the presence of subtending mature leaves are necessary for floral initiation (Scaramuzzi 1953 and Schumacher 1962 in Luckwill 1970) and it is therefore possible that one of their contributions may be to promote the active transpiration stream and so bring in a supply of cytokinins (Luckwill 1970).

However, despite numerous investigations, neither the mode of action of daminozide nor the physiology of flower bud initiation have been clarified therefore the induction of flower buds by daminozide is not easily explained and we are left to theorise.

3.4.2 Influence of chemical treatments on fruit set and shoot growth.

Because the physiology of flower initiation is not totally understood, the reasons why fruit bud numbers can be increased, with or without a concurrent change in shoot growth are difficult to identify. However, because more is known about fruit set, development and retention and the forces acting upon these, it is perhaps easier to determine the ways in which plant growth regulators influence them.

Fruit set itself is a physiological phenomenon during which, firstly pollination and then the subsequent fertilisation initiate a metabolic gradient between the fruitlet and the tree, attracting nutrients to the former (see Crane 1964). The more ovules sucessfully fertilised, the more seeds are initiated and the greater is the fruitlets 'pulling power' to compete for nutrients. Only by adequate fertilisation can the fruit of most apple cultivars develop. It is therefore desirable that the flowers themselves should be as capable as possible of achieving fertilisation. The term 'floral strength' has been used to express the chance of a flower to develop into a fruit under average circumstances and although perhaps physiologically meaningless, is a horticulturally useful term (May 1972). This strength has been reported to be visually expressed as bold flowers standing proud of the cluster leaves (Abbott 1970), with good colouring, with strong receptive styles and with ovules which remain healthy for several days (Williams 1965). These flowers may possibly have increased levels of metabolic activity enabling successful competion for nutrients even prior to pollination. They may also have the advantage of being

able to support the rapid and successful fertilisation of several if not all of their ovules. Once fertilised and initially 'set' the fruitlet must continue to attract nutrients in order to grow and develop, a process carried out in competition with the growing shoot tips (Abbott 1960, Quinlan and Preston 1971). Around flowering time, the vegetative buds burst and a period of rapid shoot growth commences. Auxins produced in the tip are thought to attract nutrients towards it (Smith and Wareing 1966); the faster the growth rate the more auxin is produced and the stronger the tip acts as a 'sink'. Within their developing seeds fruitlets also produce auxin which attract metabolites to them and therefore the two processes of shoot growth and fruitlet development are in direct competition. Although Abbott (1984) showed that the absolute number of seeds within a fruit was not necessarily crucial - a fruitlet with one seed remaining attached and showing equivalent growth to fruitlets with ten seeds - what is important is the degree of competition for resources which exists. A many seeded fruitlet will be able to attract nutrients even in the presence of strongly growing shoots or many other fruitlets. A few-seeded one may compete unsucessfully, suffer nutrient deficiency, seed abortion and consequently abscind (Abbott 1984). Practices which inhibit strong growth at this time can greatly enhance fruit set (Abbott 1960, Quinlan and Preston 1971).

Although the period during fruit set and initial rapid shoot growth may be the most competitive, some degree of competition continues until shoot growth ceases.

Within the experiments described in this chapter it was seen that certain treatments could increase the proportion of flower clusters which set fruit and also the proportion of fruitlets which were retained until final set assessment. Within some treatments shoot growth during the year of fruit production was also affected, in others it was not.

Considering the daminozide treatments first, examination of the summarised results (Table 3.4.2.1) shows clearly that effects on fruit set were highly affected by variety. This difference in varietal response to growth regulators has been seen many times (Veinbrants 1972, Grauslund 1974) but little is known about the reasons for it.

Within Cox/M9, daminozide did not alter the proportion of buds which produced fruit, therefore the trees which had produced more fruit buds (those sprayed in June) also had increased numbers of fruitlets initially set. However, after this, August daminozide application increased the proportion of initially set fruit which were retained until final set and therefore the end result was that although each time of daminozide application had acted upon a different component of fruit production, both had resulted in increased numbers of fruits present at final set.

Although within Cox/M9 only shoot number was affected by June daminozide application, the shoot number, length and consequent total growth were decreased by August application. Therefore it would seem that the reduced growth during 1986 following the August 1985 daminozide application had resulted in decreased competition operating against the developing fruitlets. As such, a larger proportion of these were retained until final set.

Table 3.4.2.1 Summary of treatment effects on shoot growth during the year of treatment (1985), the year following treatment (1986) and the components of fruit production in 1986. '⬆⇧' and '⬇⇩' indicate that the obtained value was significantly more or less than on controls, '⬆⬇' and '⇧⇩' refer to June and August applications respectively. Treatments were applied to (a) Bramley/M26 rootstock, (b) Cox/M9 rootstock and (c) Cox/MM106 rootstock.

(a) Bramley on M26 rootstock

Treatment	1985 shoot number	1985 mean length	1985 total growth	1986 shoot number	1986 mean length	1986 total growth	1986 bud number	1986 % set	1986 initial set	1986 % initial → final set	1986 final set
Paclobutrazol		⬇	⬇	⬇⇩	⬇⇩	⬇⇩	⬆⇧	⬆	⬆⇧		⬆⇧
Daminozide		⬇	⬇				⬆				
Tying							⬆	⬇			⬇
Tipping		⬆	⬆					⬇			

(b) Cox on M9 rootstock

Treatment	1985 shoot number	1985 mean length	1985 total growth	1986 shoot number	1986 mean length	1986 total growth	1986 bud number	1986 % set	1986 initial set	1986 % initial → final set	1986 final set
Paclobutrazol							⬆	⇧	⬆		⬆
Daminozide				⬇⇩	⇩	⬇⇩	⬆		⬆	⇧	⬆⇧
Tying											
Tipping										⬆	

(c) Cox on MM106 rootstock

Treatment	1985 shoot number	1985 mean length	1985 total growth	1986 shoot number	1986 mean length	1986 total growth	1986 bud number	1986 % set	1986 initial set	1986 % initial → final set	1986 final set
Paclobutrazol								⇧	⇧		⇧
Daminozide		⬇	⬇	⬇⇩	⇩	⬇⇩		⬆⇧	⬆⇧		⬆⇧
Tipping											

Within Cox/MM106, daminozide had not increased the number of fruit buds produced in 1986 but application at either time had increased the proportion of these which set fruit, thereby increasing the actual number of fruits set both initially and finally. Concurrent with this was a marked reduction in total shoot growth. Unlike Cox/M9, the percentage of fruit retained until final set was not affected by any treatment.

Thus within the daminozide treatments we have the situation where changes in the components of fruit production occur alongside changes in vegetative growth. Therefore, perhaps the increased fruitlet set and retention observed can be explained in terms of decreased competitive pressures from shoot growth.

However, as regards the paclobutrazol treatments, the situation is very different. In one case (Bramley) increased percentage set occured concurrent with extreme inhibition of shoot growth (June application), but in two other cases (Cox/M9 and Cox/MM106 rootstocks) where a similar increase in percentage set occured, there was no associated inhibition of growth. That growth and fruit set are antagonistic is in no doubt, but it is not clear how paclobutrazol can increase the proportion of buds which set fruit without affecting shoot growth. Of course because the measurements of shoot growth were not detailed enough to identify any differences in the timing of growth throughout the season, it is possible that shoots on the paclobutrazol treated trees started growing only after the period of flowering and fruit set was over. However, no visual indication of such differences was seen. The alternative explanation is that paclobutrazol somehow enhanced the inherent quality of fruit buds such that they themselves were better able to cope with competition for resources. Of interest here is the number of seeds set per fruit. Generally it might be expected that the more seeds which a fruit contains, the more auxin it will be producing and therefore the more effective a 'sink' for resources it will represent (Crane 1964). Although no differences in seed number were found within the fruit from Bramley or Cox/MM106, treatment with paclobutrazol or daminozide in June significantly increased the seed number in fruit on Cox/M9. This might suggest that these treatments encouraged a higher level of fertilisation and healthy embryo development within flowers such that the fruits produced were better able to attract metabolites, even though they were in competition with unaltered rates of shoot growth. If this is a reflection of higher levels of successful fertilisation, this may possibly have been induced by late, or slow shoot growth around flowering. If so, the flowers themseves would have had less competition to contend with and may therefore have remained viable for an extended time, thus maximising their chances of pollination and fertilisation.

3.4.3 Effects of cultural treatments on shoot growth and flower initiation.

The applied cultural treatments also affected shoot growth and flower initiation but to a lesser extent than the chemical treatments.

Considering the effects of branch orientation first, it was seen that branches which were maintained in a horizontal orientation often had reduced growth compared to those in a more upright position. This was not seen in the 1-year-old trees (Experiment 3) where although branches were tied down, shoot tips grew in a vertical direction or (in Experiment 4) where shoot tips of tied down Discovery branches grew in a similar manner. This is in agreement with the results obtained by Mika (1969) who trained branches to a downward orientation but allowed tips to grow upright and found that the total amount of shoot growth produced was unaffected. However in the experiments described here, when growing shoot tips were maintained in a horizontal position, the mean shoot length of Cox/M9, Cox/MM106 and Bramley/M26 was decreased compared to treatments where shoots grew more upright.

This reduced growth of horizontal branches has been observed in orchards since commercial apple growing began (Knight 1803, Swarbrick 1929), and has been demonstrated in numerous experiments (Wareing and Nasr 1958, Kato and Ito 1962, Tromp 1970, 1972, 1987). It has been attributed both to changes in distribution, either within individual shoots or between the different shoots on the tree, of hormones (Tromp 1982) and/or nutrients (Smith and Wareing 1966), and to changes in their production (Kato and Ito 1962, Robitaille and Leapold 1974).

Alongside the observed effects on shoot growth, were several changes in flower production. Generally fruit-bud number was increased in situations where shoot growth had been decreased (i.e. Bramley and Cox/M9 in Experiment 2). But there were also situations where reduced shoot growth was not accompanied by any increase in fruit bud number (Cox/MM106 in Experiment 2) and also where increased bud numbers occured with no decrease in shoot growth (Discovery in Experiment 4).

Here, as with the results of chemical growth regulator applications, we have evidence to suggest that shoot growth and flower initiation are not inevitably linked, and that it is possible to influence one without affecting the other.

Why growth should be inhibited by a horizontal orientation is unclear but Kato and Ito (1962) demonstrated that horizontally growing apple shoot tips had a lower IAA content than did vertical growing ones suggesting that gravity might affect growth through regulation of endogenous hormones. As Tromp (1972) pointed out, this also supports the finding of Luckwill (1968) that the supply of nutrients to the shoot apex is controlled by auxin produced at the tip meristem. Although Fulford *et al.* (1968) found that the tendency to import auxin to the shoot tip was less pronounced in vigorously growing shoots, this does not necessarily contradict the theory of vigorous shoots having higher auxin levels; they may simply import less because they are already producing sufficient.

Similarly, how shoot orientation in relation to gravity may affect flower bud formation is obscure though of course the possibility of an indirect effect through the release of lateral buds

from apical dominance, resulting from reduced shoot growth, must be considered. Wareing and Nasr (1958) first recognised that gravitational effects may be important in the apical dominance of woody plants and later Wareing and Nasr (1961) concluded that the apical dominance of a shoot could only be fully manifested when that shoot occupied a vertical position. Thus if apical dominance is reduced by a horizontal position then the actual effects of branch bending may be very similar to those induced by the chemical growth regulators. That is, if they somehow slow growth and therefore reduce apical dominance, the lateral buds below the tip will be able to increase their internal activity and rate of development such that a greater proportion of them reach sufficient complexity for floral induction.

This leaves us with the question that if a horizontal orientation reduces apical dominance, by what method does it achieve this?

Apical dominance has been suggested to be one of the main factors controlling flower initiation (Nasr and Wareing 1961), and Luckwill (1968) stated that this dominance took two forms - one where the activity within lateral buds is inhibited by that within the shoot tip, and the other whereby the growth of lower shoots is inhibited due to the presence of strongly growing upper ones.

Of these, Wareing and Nasr (1961) showed that the former is only fully manifested when a shoot occupies a vertical position. Thus, alteration of branch angle from upright to horizontal throughout a tree will have a marked effect on the degree of apical dominance operating. Firstly, apical dominance within each branch will be reduced thereby allowing greater activity within lateral buds. Secondly, because the apical dominance, and consequent rapid growth of upper shoots is reduced, their inhibitory effect on the growth of the lower shoots will also be lessened.

In a horizontal oat coleoptile, auxin redistribution results in a greater concentration on the lower side than on the upper one (Dolks 1930) and use of ^{14}C-IAA has shown that a similar redistribution may occur in woody plants as well (see Wareing and Nasr 1961). Auxin redistribution within each horizontal branch would further lower the levels in the buds on the upper side thus allowing them to become more active.

Decreased gibberellin activity has also been implicated as being one of the effects of altering branch orientation, Tromp (1982) finding that spraying trees with this hormone at the same time as training branches into horizontal positions could negate the effects of the orientation treatment.

In the recent experiments where certain treatments reduced mean shoot length, but did not increase flower bud number (Cox/MM106 in Experiment 2), total shoot growth over the trees was not reduced. Any reduction in apical dominance associated with the lesser growth on these branches may simply not have been enough to allow the lateral buds to reach sufficient complexity for flower initiation to occur and may have been counteracted by the total

growth remaining the same overall. However, it still does not explain how in Experiment 4 Discovery trees with horizontally trained branches produced more flower buds but no less shoot growth than did trees with upright branches.

Mullins (1965) found that although horizontally orientated branches had less extension growth than upright ones, overall shoot growth was the same for trees with vertical branches. He also found that although weakly growing horizontal branches formed more flowers than more vigorously growing ones, the number formed on the tree as a whole was unaffected. Because in the present experiments, both shoot growth and flower number were assessed on a whole tree basis, there is no information regarding whether or not more buds were formed on the weaker growing shoots compared to the strongly growing ones or whether there really was no relation between strength of shoot growth and flower initiation.

Alternatively the results may agree with the those of Tromp (1970) who found that turning trees into a horizontal position after growth had stopped could still increase the number of fruit buds formed, indicating that orientation must have a direct, and enhancing, effect on floral initiation. But this is contradicted by the results of Experiment 4 where firstly, the earlier in the season at which branches were tied horizontally, the more fruit buds were formed the following year and secondly, tying branches horizontally after growth had stopped did not influence fruit bud number. It is possible though that response to branch orientation is highly dependent on the individual situation and the age, nutrient status and variety of the tree used.

Compared to branch orientation, shoot tipping had very few effects on shoot growth and flower initiation. Generally it was seen that although shoot growth had been enhanced by removal of shoot tips in June, no effects on the number of flower buds formed were seen.

3.4.4 Effects of cultural treatments on fruitlet set and retention and shoot growth.

The varying effects of treatments on fruit set and retention were rather confusing and compounded by wide differences in response between cultivars.

Considering branch orientation first; in Experiment 3, Cox on both M9 and MM106 on which branches had been trained horizontally, set increased numbers of fruit per hundred flower clusters compared to trees with more upright branches. Although no differences were seen in the percentage of initial set subsequently retained until final set assessment, trees with horizontal branches had more fruit at final set assessment. Alongside this, they also had reduced shoot growth. Bearing in mind the intense competition between shoots and fruit set (Abbott 1960, Quinlan and Preston 1971) this situation might have been anticipated. However, within Bramley (Experiment 2) tying branches down in June was associated with decreases in percentage set and percentage fruitlet retention the following year. Although fruit bud number had originally been increased by this treatment, the subsequent poor set and retention negated this. Given that in other treatment situations increased bud number had been trans-

lated into increased numbers of fruits initially set and retained until final set assessment (Discovery in Experiment 4) it is strange that this should not have occurred here. Although no differences in shoot growth either before or during the year of cropping were observed, it must appear that either the flowers on these trees were of inherently poorer quality than those within other treatments or that the trees have a finite 'carrying capacity'. If the latter was true, then resource limitation may prevent increased fruit set and retention even when more buds are available. This did not appear to be the case within Cox/M9 in which paclobutrazol or daminozide treated trees both had increased numbers of fruit buds which were then translated into increased numbers of fruits set initially and retained. However, big differences in varietal response have been seen before so perhaps Bramley are more resource limited than are Cox. Evidence in support of this is that although daminozide application to Bramley had also increased fruit bud number, no increase in the number of fruit set was achieved. Only paclobutrazol application (which severely reduced shoot growth in the year of cropping) resulted in increased numbers of fruitlets being formed. Perhaps therefore Bramley are largely affected by carrying capacity and only with reduced shoot growth can increased yields be obtained.

It is confusing that shoot tipping should decrease percentage fruit set in Bramley yet increase percentage fruitlet retention within Cox/M9. Within Bramley, unlike the Cox, this treatment increased shoot growth the year prior to cropping: it is therefore possible that this increased growth detrimentally affected flower quality. There are many references in the literature to the fact that flower initiation occurs around the time of shoot growth cessation (Barnard and Read 1932, Huang 1987), therefore it might be expected that if the increased growth induced by tipping was caused by a longer growing period, then flower initiation would have occurred later on these trees than on those where growth stopped earlier. Abbott (1970) showed very clearly that the length of time between inception of flower buds and winter dormancy has a considerable influence on flower quality the following year. He found that where only a short time elapsed between floral initiation and dormancy, the resultant flowers were 'weak' and set very poorly compared to those which had had a longer developmental period. Therefore the reduced conversion of flowers into fruit on branches which had been tipped can perhaps be explained by them having had a delayed initiation due to maintained shoot growth and therefore a shortened developmental period.

It is more difficult to explain the increased retention of fruitlets which result from tip removal in August of the previous year. As previously discussed, the main factors affecting fruitlet retention are the forces of competition operating at the time. Unless shoot tipping had a delayed effect on shoot growth it is hard to explain its effect on retention. Even if the absence of the terminal bud shoot growth delayed the start of shoot growth in the following year, this would be expected to affect initial fruit set rather than fruitlet retention, which would presumably be accompanied by the resumption of strong shoot growth.

Chapter 4. Cluster development prior to flowering: influence of tree age, wood age and branch orientation.

4.1 Introduction

In order that any apple flower can set fruit it must be able to support pollen germination and pollen tube growth, and must have ovules which remain healthy long enough for the pollen tubes to reach them. For this to occur, flowers must be of certain minimum 'quality'. The idea of floral 'quality' or 'strength' has been described by May (1972) as 'a physiologically meaningless but horticulturally useful concept which expresses the chance of a flower to become a fruit under average environmental conditions'.

Throughout the literature there are descriptions of flowers and/or clusters being either 'poor quality' and 'weak', or 'good quality' and 'strong'. These have usually been visual assessments based on one or a combination of flower size and number, colouration, or leaf size or number. Williams (1965) described 'strong' flowers as being large, more vigorous and produced from plumper fruit buds and showed them to have a greater ovule longevity and stigma receptivity than 'weak' flowers. Goldwin (1978) visually assessed 'good quality' flowers by their larger size, strong pedicels and bold pink colouration. Similarly Abbott (1984) associated good flower 'quality' with pink petals, dark green leaves and large cluster size. 'Good quality' flowers are generally thought to set fruit more successfully than are 'poor quality' ones.

That flower size can be associated with setting ability has been shown experimentally by several workers. Abbott (1971) found poor fruit set associated with small flowers (which had been induced by warm spring temperatures) and Ferree and Rom (1984) found poor set on 'weak' spurs bearing small flowers. Similarly Hill-Cottingham and Williams (1967) found that large flowers (induced by nitrogen treatments) were 'strong' and set well.

However, it is not only the flowers which are important. They, and the fruit resulting from them are largely composed of photosynthates and organic N complexes, therefore it follows that the presence of sufficient healthy leaves are required to supply these.

Although it is established that the early spring growth of the buds on deciduous trees utilise stored reserves (Priestley 1960), primary leaves are of crucial importance in fruit set (Ferree and Palmer 1982), being both the main centres of photosynthate production and also important in the hormonal balance of the tree. These leaves supply photosynthate for use by the developing flowers from as early a stage as 'pink bud' (Quinlan pers com) and removal of spur leaves during flowering has been shown to reduce fruit set (Llewelyn 1968, Ferree and Palmer 1982). Hansen (1971) showed that the greater part of the growth of new fruit depends on current leaf photosynthate and Ferree and Palmer (1982) showed that during this early growth, fruits are highly dependent on the leaves of their own spurs rather than being able to receive photosynthate from elsewhere.

Chlorophyll is also of importance being required for the first stage in the transformation of light energy to chemical energy. Low chlorophyll levels may result in decreased in photosynthesis and perhaps a sub-optimal supply of photosynthates to the developing flower.

A balanced supply of minerals is needed in order to maintain a large physiologically active leaf surface (Kozlowski 1971) and much evidence has shown that minerals are involved in all major phases of reproductive growth including initiation of flower primordia and fruit set. The key element is nitrogen, but potash, magnesium and boron are also important for reproductive growth.

Where a deficiency of a given mineral element occurs, fruit set may be inhibited (Kozlowski 1971). Conversely, application of nitrogen can increase fruit set (Fisher *et al.* 1948, Williams 1965, Delap 1967) even when nitrogen levels were already judged to be high (Yogaratnum and Greenham 1982) and sprays of magnesium (Greenham and White 1959), zinc (Ford 1970) and boron (Davison 1971) can all increase fruit set levels above controls.

Consequently, in order to elucidate what the factors contributing to 'strong' or 'weak' flowers might be, a study was made of three separate situations in which flowers, although regularly produced, often set very poorly, thus suggesting the flowers to be of poor 'quality'. These were compared with others borne in situations usually expected to set fruit well.

The three situations chosen were:

a) 1-, 2- and 3-year-old wood within a branch - flowers on 1-year-old wood often being reported to set badly (May 1972),

b) horizontal and vertical branches - fruit production on the latter often being much poorer than on the former (Preston 1974),

c) various ages of tree (from 2- to 12-years-old) - flowers on young trees being reported to set less well than do those on older trees (Forshey 1978).

Within each situation cluster development during the period from 'bud burst' to 'full bloom' was monitored for flower weight, leaf weight size and thickness, mineral and chlorophyll content and the amount of carbon assimilated by the flowers at selected stages.

.2 Materials and methods

4.2.1 Field material and experimental design

To investigate whether tree age, wood age and/or branch orientation might affect cluster 'quality' through changes in morphology and/or nutritional status, fruit bud development was monitored from 'bud burst' to full bloom in the following situations;

(i) 1-, 2- and 3-year-old wood within the same branch,

(ii) Horizontal or vertical branches within the same trees,

(iii) 2-, 3-, 4-, 6- and 12-year-old trees.

The trees were all Cox and are described in Section 2.2.1 and Table 2.2.1.

To assess 'between wood-age' differences, clusters from 3-, 2- and 1-year-old wood within horizontal branches on 4-year-old trees were examined. To assess branch angle effects, clusters on 2-year-wood growing on horizontal or vertical branches within 4-year-old trees were similarly examined. When assessing 'age of tree' effects, clusters on 2-year-old horizontal wood within the various ages of tree were used.

In all situations, clusters were harvested at several predetermined developmental stages, care being taken to select clusters which conformed exactly to the criteria required (Table 4.2.1). Samples were collected when approximately 50% of the buds in the relevant situation were at the required stage, and were harvested from midway along the relevant section of wood. In the majority of comparisons bud development progressed synchronously, allowing all harvests for a particular stage to be made on the same date. Exceptions to this were buds on the youngest trees and those on on the previous years' extension growth. These were delayed by approximately 3 and 5 days respectively.

At each collection, 12 clusters per treatment (two from one branch unit on six trees) were harvested and transported back to the laboratory within sealed polythene bags, resting on crushed ice within a cool box in order to minimize respiratory losses.

From the 'early green cluster' stage onwards, buds were separated into leaves and flowers. Samples were weighed, leaf area measured (using a Li-Cor portable area meter), and flowers counted. Two subsamples of leaf tissue were removed; one for chlorophyll analysis, the other for anatomical examination. The remaining samples were reweighed then oven dried at 80°C prior to analysis of mineral content.

Parallel to this experiment, at four collection times ('early green cluster', 'pink bud', 'first flower' and 'full bloom') the uptake and distribution of $^{14}CO_2$ by spur leaves and flower buds was measured. Clusters on 2-, 4-, 6- and 12-year-old trees were used with or without prior spur leaf removal. On the 4-year-old trees, clusters on different branch orientations were examined in the same way.

4.2.2 Preparation of material for anatomical examination

Segments of leaf tissue (approximately 10mm x 3mm) were excised from the central lamellar area and fixed in 4% formalin in 0.1M phosphate buffer at pH 6.8 *in vacuo* for 24 hours. After storage for 24 hours or longer, samples were rinsed in water, dehydrated in an ethanol/propan-1-ol series and finally embedded in paraffin wax.

Sections 10µm thick were cut on a rotary microtome and mounted on microscope slides using Haupts' adhesive and 4% formaldehyde. After drying overnight at 30°C, slides were stained with toluidine blue, rinsed, then dewaxed in xylene. Sections were examined using a projection microscope, screen measurements being calibrated by use of a slide graticule. Using leaves collected at 'early green cluster', 'pink bud', and 'full bloom' from 2-, 3- ,4-, 6-

Table 4.2.1. Phenological stages of bud development at which sample collections were made.

style of bud development		morphological characteristics
(i)	break	separation of the bud scales
(ii)	burst	leaf tips just showing, less than 1 cm protruding
(iii)	mouse ear	2-3 leaves separate from fruit bud
(iv)	early green cluster	2-4 leaves bent outwards
(v)	late green cluster	more than half the flower buds separated
(vi)	pink bud	half or more flower buds show colour
(vii)	first flower	lateral petal dome larger than the ovary, king flower open
(viii)	full bloom	all lateral flowers open

(adapted from Hamer 1980)

and 12-year-old trees, 10 sections per treatment were examined and measurements of leaf thickness made.

4.2.3 Chlorophyll analysis

Approximately 0.5g. of finely ground chopped frozen tissue was extracted in cold (-20°C) 80% acetone for 24 hours after which the optical density at both 645 and 663 nm was determined. Chlorophyll content was derived from the equations;

Chlorophyll a = $(12.7 \times A_{663}) - (2.7 \times A_{645})$ mg/l

Chlorophyll b = $(22.9 \times A_{645}) - (4.7 \times A_{633})$ mg/l (Arnon 1949)

Results were then converted to µg chlorophyll a and b per mg leaf fresh weight.

4.2.4 Mineral analysis

After oven drying at 80°C for 96 hours, samples were Kjeldahl digested; 100mg plant tissue being heated at reflux for 90 minutes with 2ml Kjeldahl digestion mixture ($1gl^{-1}$ selenium powder in H_2SO_4) and 1ml hydrogen peroxide. On cooling, samples were made up to 20 ml and the solutions used for analysis. Nitrogen and phosphorus were determined colorimetrically; potassium, calcium and sodium by flame emission spectroscopy, and magnesium by atomic absorption spectroscopy (AAS). Samples were then ashed at 470°C, dissolved in dilute nitric acid and the levels of manganese, and zinc determined by AAS.

4.2.5. Feeding and recovery of ^{14}C carbon

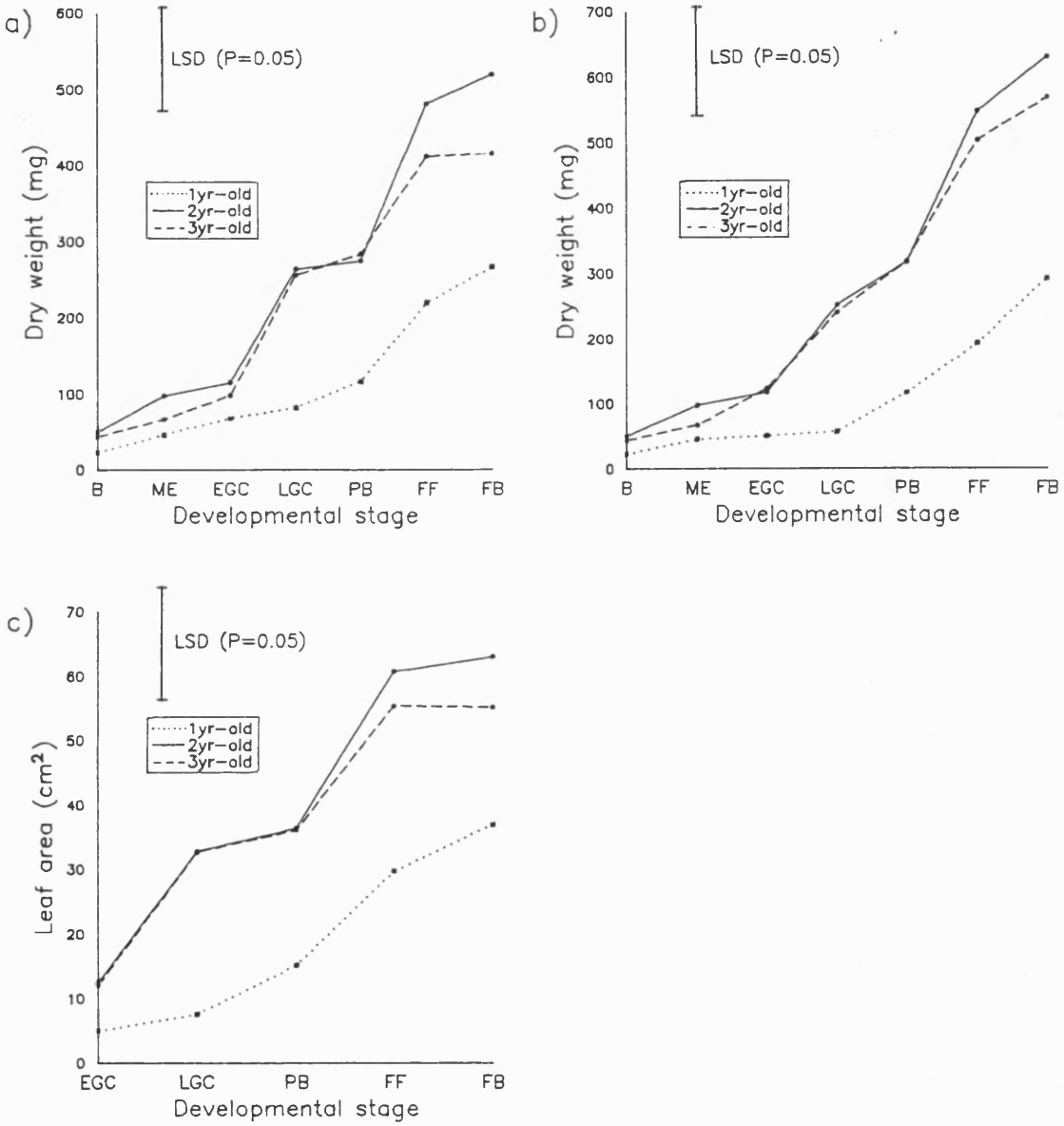
Clusters either with or without spur leaves were fed $^{14}CO_2$ for one hour between 9 and 11 a.m. using the methods of Quinlan (1969). After 24 hours, treated clusters were harvested and lyophylised. Dried samples were combusted in a Harvey Biological Material Oxidiser, the resulting CO_2 being trapped in scintillation cocktail based on toluene containing PPO scintillant. Radioactivity was determined by liquid scintillation spectroscopy (Beckman LS7800): cpm automatically corrected for background and quenched to give dpm.

4.3 Results

4.3.1 Cluster development

By April 3rd 1985, approximately 50% of spur buds on most ages of Cox trees had broken, scale loosening being visible. By April 8th these were at 'burst' with some scales being shed and the bud emergent underneath.

Bud burst occurred synchronously on 2- and 3-year-old wood but was delayed on 1-year-old wood, where clusters reached equivalent stages about 5 days later. Within individual trees, clusters borne on 2- or 3-year-old wood had very similar growth patterns until 'pink bud' after which those on 2-year-old wood increased leaf area and weight more rapidly than did those on 3-year-old wood, being significantly larger and heavier by 'full bloom' (Figure 4.3.1.1). Clusters from 1-year-old wood were consistently smaller and lighter than were those from older



Figures 4.3.1.1a-c

(a) Bud and leaf weight
(b) bud and flower weight
(c) leaf area

of clusters borne on 1-, 2- and 3-year-old wood; at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.

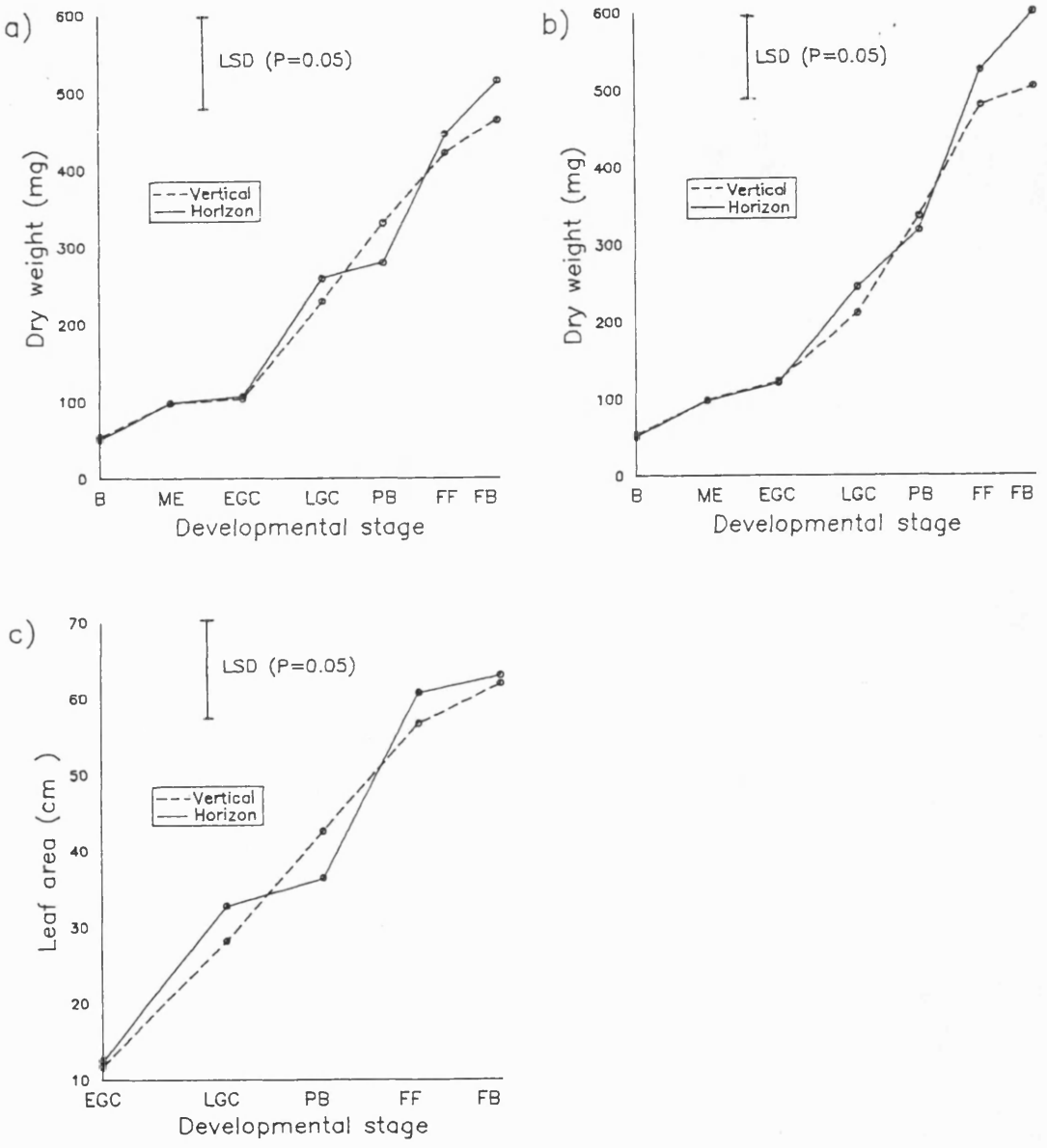
wood, the differences becoming most pronounced after 'early green cluster' when their rate of growth increase was markedly less than that of clusters from older wood. This was such that at 'full bloom' flower buds and leaves from 1-year-old wood weighed approximately half that of those from older wood.

Within the two branch orientations examined, during initial cluster development the size and weight of cluster leaves borne on horizontal branches was almost identical to that of those borne on vertical branches (Figures 4.3.1.2a and b). Initially the increase in flower weight during this period was also similar but after 'pink bud' flowers from horizontal branches increased in weight more rapidly than did those from vertical branches (Figure 4.3.1.2b) such that by 'full bloom' flowers within clusters on horizontal branches weighed approximately 20% more than did those from vertical branches.

Although bud 'burst' and development occurred synchronously on most ages of tree studied (i.e. 3-, 4-, 6- and 12-year-old), buds on 2-year-old trees developed more slowly and reached equivalent stages 3-4 days later. At both 'burst' and 'mouse ear' stages, buds collected from the youngest trees, although at an apparently identical developmental stage to the ones collected from older trees, were consistently and significantly lighter (Figure 4.3.1.3a), being only half the weight of the buds from older trees. Buds collected from 'green cluster' onwards were separated into leaf and flower components. Of those from the 3- to 12-year-old trees, few differences were apparent in leaf area or weight, or total flower weight during the period until 'first flower', (Figures 4.3.1.3a and b). After this stage the weight of leaves from the 3- and 4-year-old trees increased at a greater rate than did those from the 6- and 12-year-old trees such that those from the former were significantly heavier than the latter at 'full bloom'. Weight of leaves from the 2-year-old trees increased very little after 'pink bud', such that at 'full bloom' they were on average, less than half the weight of those from the 3- and 4-year-old trees.

In clusters from all ages of tree, the weight of flower buds increased steadily between 'green cluster' and 'pink bud' . After this stage, buds on the 4-, 6- and 12-year-old trees continued to increase in weight but those on the 2- and 3-year-old trees increased weight only slightly after 'pink bud' such that at 'full bloom' they had only half the weight of flowers on the older trees. Flower weight on the 2-year-old trees actually decreased between 'pink bud' and 'first flower' due largely to premature abscission of individual flowers (Table 4.3.1.1). This abscission continued between 'first flower' and 'full bloom' although total bud weight remained relatively constant, indicating that some growth of individual flowers must therefore have continued.

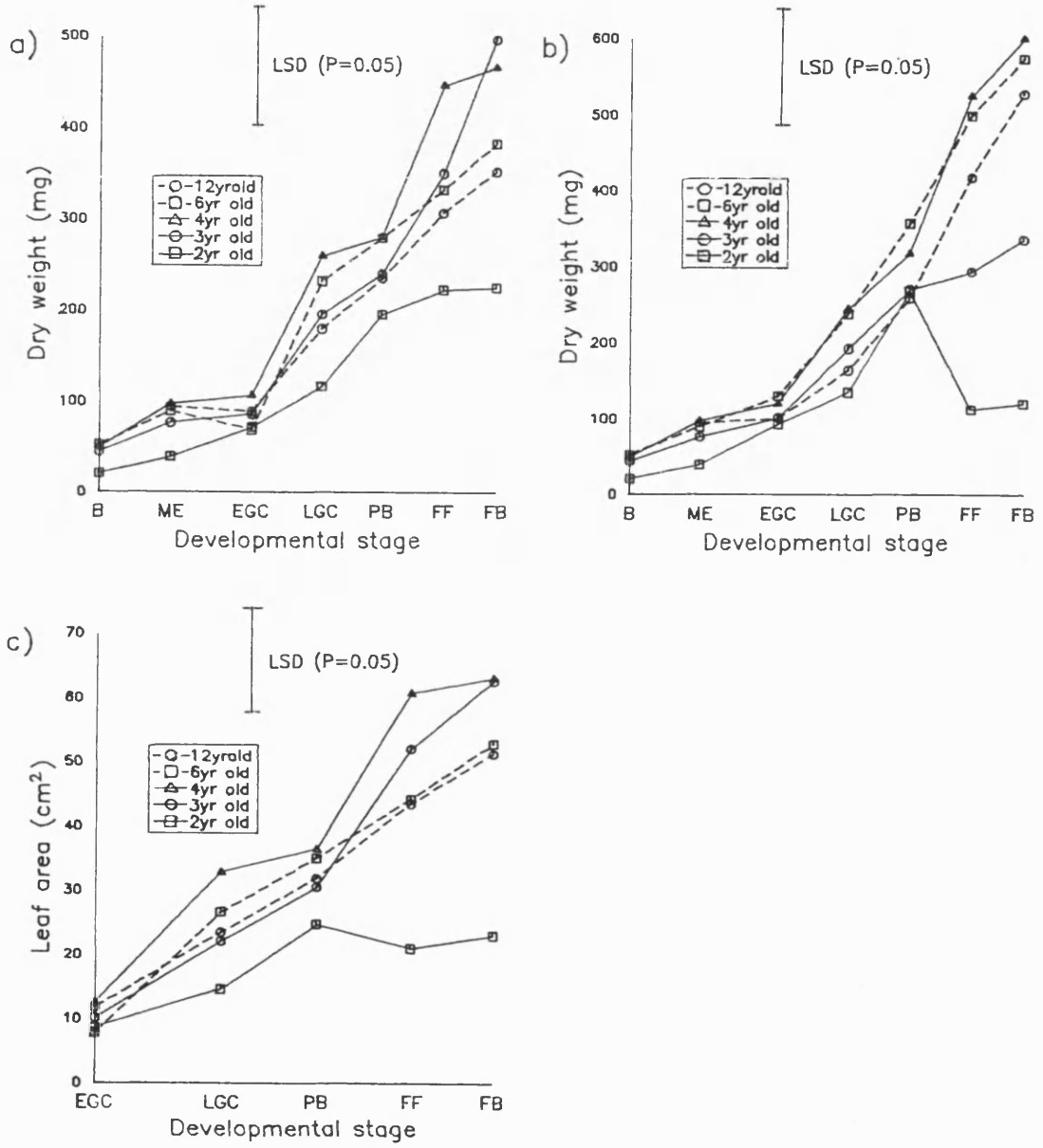
Leaf area of clusters from all ages of tree increased steadily between 'early green cluster' and 'first flower'. After this stage those on the older trees continued to expand, whilst the leaf area of clusters from 2-year-old trees decreased slightly, presumably due to leaflet abscission, before stabilising.



Figures 4.3.1.2a-c

(a) Bud and leaf weight
(b) bud and flower weight
(c) leaf area

of clusters borne on 2-year-old wood within horizontal and vertical branches; at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.1.3a-c

(a) Bud and leaf weight
(b) bud and flower weight
(c) leaf area

of clusters borne on 2-year-old wood within various ages of tree; at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.

Table 4.3.1.1a-c Number of flowers per cluster on various (a) ages of tree, (b) orientations of branch and (c) ages of wood, at early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).

(a)

age of tree	developmental stage				
(years)	EGC	LGC	PB	FF	FB
2	5·8	6·1	6·0	5·3	4·5
3	6·0	6·2	5·9	5·9	5·6
4	6·2	6·3	6·4	6·3	6·2
6	6·5	6·4	6·7	6·5	6·0
12	6·0	6·1	6·2	6·2	6·0
S.E.D.			0·31		

(b)

branch	developmental stage				
orientation	EGC	LGC	PB	FF	FB
horizontal	6·2	6·3	6·4	6·3	6·2
vertical	6·1	6·0	6·2	6·2	6·2
S.E.D.			0·27		

(c)

age of wood	developmental stage				
(years)	EGC	LGC	PB	FF	FB
1	4·9	4·7	4·6	4·6	4·8
2	6·3	6·3	6·4	6·3	6·2
3	6·0	6·1	5·9	6·0	5·9
S.E.D.			0·29		

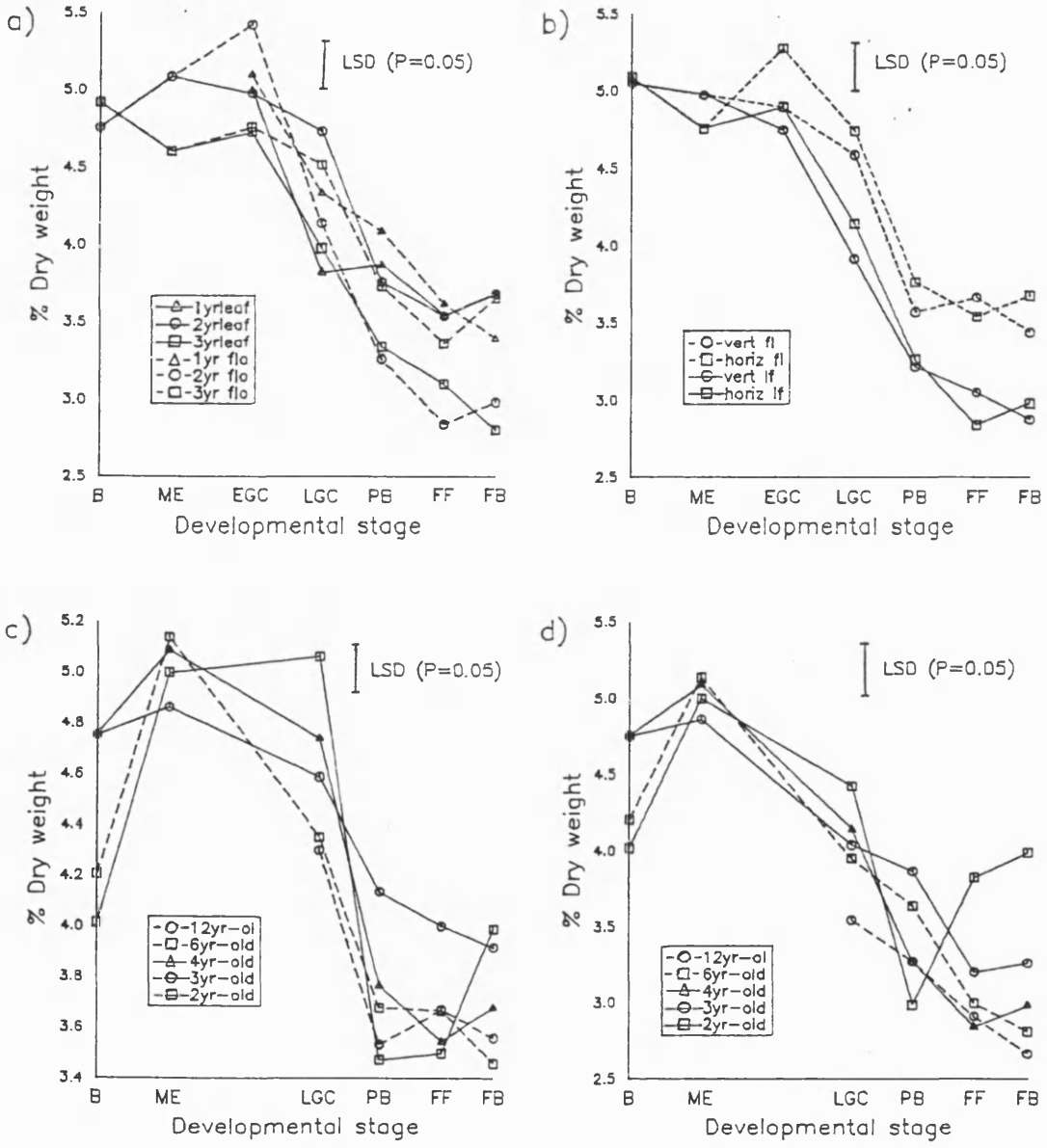
4.3.2 Mineral content of clusters

Within both flower and leaf tissues mineral concentration varied widely, both between individual clusters in the different situations under examination, and also between the various developmental stages.

In general, nitrogen content within leaf and flower tissue decreased as clusters developed (Figure 4.3.2.1). At 'bud burst' buds were about 5% (tissue dry weight) nitrogen which then decreased such that at 'full bloom' flowers were about 3.5%, and leaves about 3% nitrogen, with flowers generally having a higher concentration of nitrogen than did leaves. No concentration differences were seen between clusters on the various wood ages, and although when comparing clusters on the two branch orientations nitrogen concentrations were generally higher within those from horizontal branches, differences were not significant (Figure 4.3.2.1b). Within the clusters from the various tree ages nitrogen concentration at 'bud burst' varied slightly, being 4% and 4.2% in those from 2- and 6-year-old trees compared to 4.8% in those from 3- and 4-year-old trees (Figures 4.3.2.1c and d). Within clusters from all but the youngest trees nitrogen concentration within buds increased between 'burst' and 'mouse ear' and then declined steadily.

Clusters from the youngest trees displayed a slightly different pattern of changing nitrogen concentration during development. Within their leaves nitrogen concentration remained high (c 5%) until after 'late green cluster' and although concentrations within their flowers were initially similar to clusters from older trees, a sharp decline in concentration occurred between 'late green cluster' and 'pink bud'. After this stage the nitrogen concentration increased again such that at 'first flower' and 'full bloom' leaves from these clusters had a significantly higher concentration than did leaves from any other age of tree.

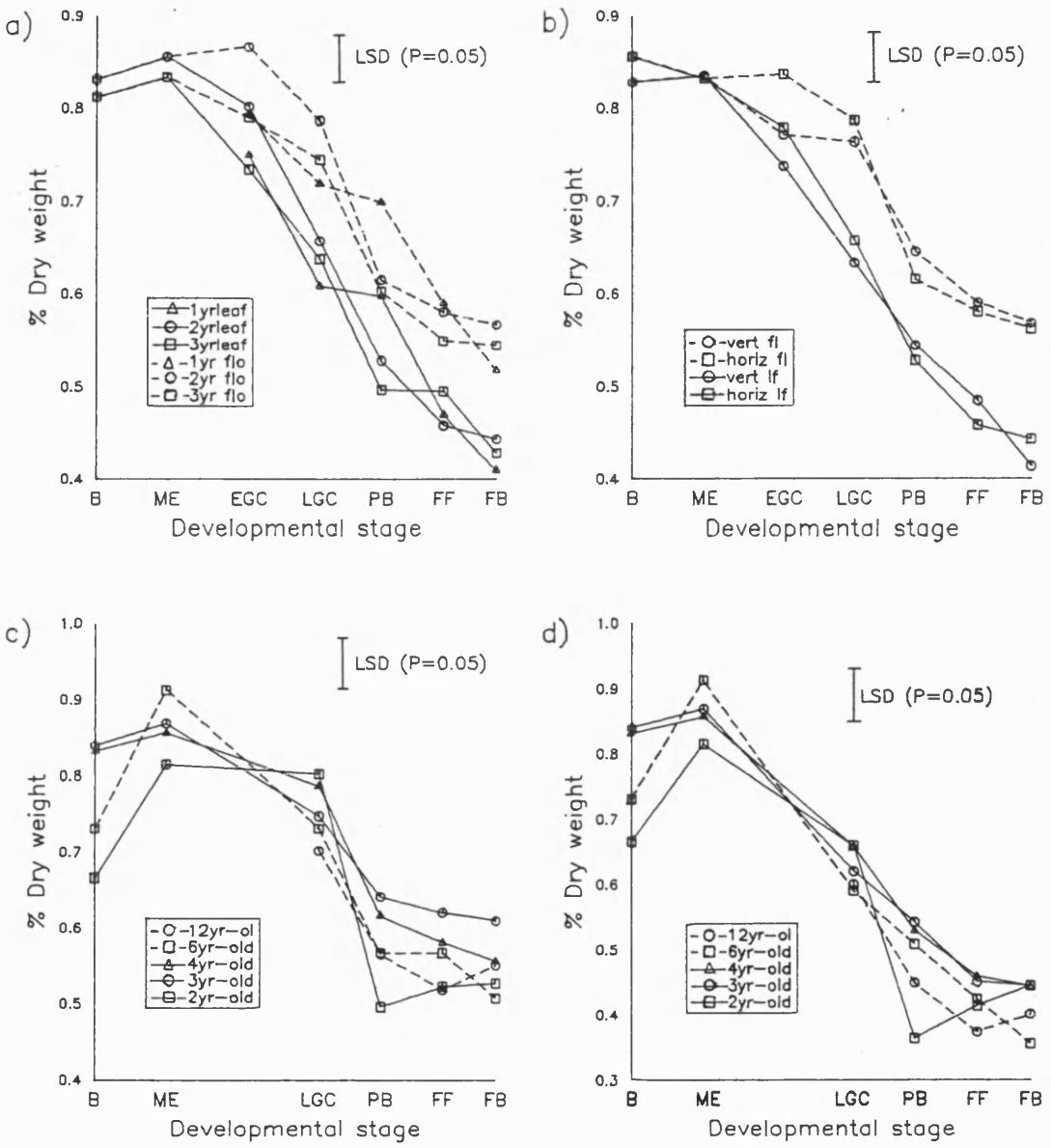
Similar to the changing patterns of nitrogen concentration within developing clusters, phosphorus concentration declined quite steadily over the whole development period (Figure 4.3.2.2). Buds at 'burst' were about 0.8% phosphorus but by anthesis this was reduced by 50% in leaves and 25% in flowers. Again flowers generally had higher concentrations than did leaves, and at 'pink bud', both flowers and leaves from clusters on 1-year-old wood had significantly higher phosphorus concentrations than did those from other wood ages although no differences were seen at any other time (Figure 4.3.2.2a). Similarly, no differences were observed between the clusters from horizontal and vertical branches (Figure 4.3.2.2b). Within clusters from the different ages of tree a slightly different pattern was seen in that those from 2- and 6-year-old trees had a slightly lower phosphorus concentration at 'burst' (Figures 3.3.2.2.c and d) which then rose rapidly such that at 'mouse ear' they were very similar to those from the other ages of tree. Few differences were seen between flower and leaf tissue but in clusters from 2-year-old trees concentrations dropped quite rapidly at 'pink bud' before rising again towards 'full bloom'.



Figures 4.3.2.1a-d
Nitrogen concentration (% dry weight) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.2.2a-d
Phosphorous concentration (% dry weight) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

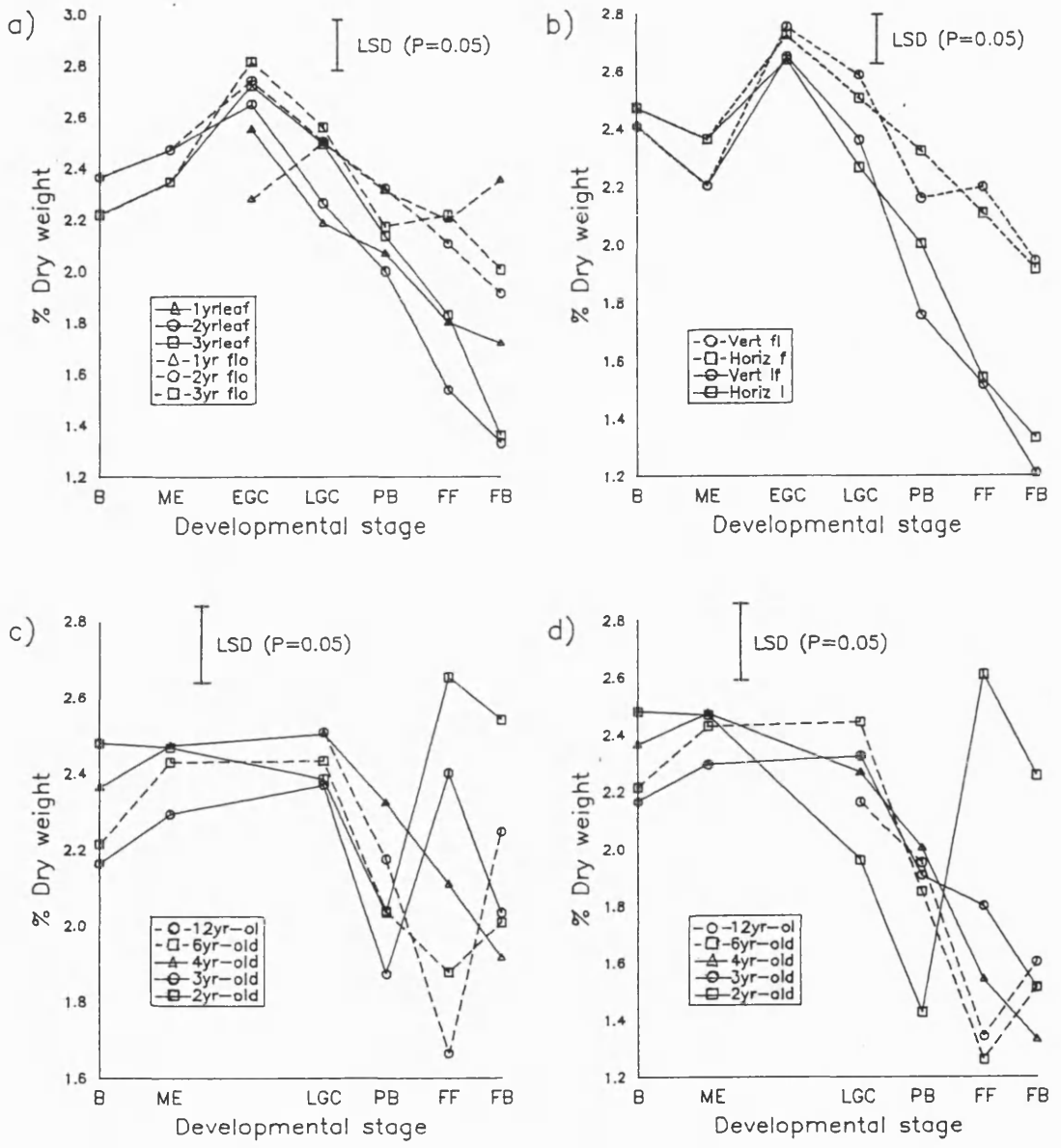
at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, ie. leaves plus flowers.

Unlike nitrogen and phosphorus concentrations, those of potassium did not decline immediately after 'burst' but rather, in clusters from all ages of wood they increased slowly until 'early green cluster' before declining as development progressed to 'full bloom' (Figure 4.3.2.3a). Again flowers had higher concentrations than did leaves, and although no differences were seen in these between the clusters from either the two branch orientations or from 2- and 3-year-old wood (Figure 4.3.2.3b), those from 1-year-old wood had significantly higher potassium concentrations at 'full bloom' than did those from older wood. At 'first flower' and 'full bloom', potassium concentrations in both leaf and flower tissue were higher in clusters from 2-year-old trees than in those from any other tree age (Figures 4.3.2.3c and d).

Compared to nitrogen, phosphorus and potassium, calcium displayed a different pattern of changing concentration; within both leaves and flowers this increased steadily during cluster development. This pattern of changing concentration was initially very similar within clusters from all three wood ages (Figure 4.3.2.4a) and levels were only slightly higher in the leaves compared to the flowers but again concentrations within flowers from 1-year-old wood were very much higher than within clusters from other wood ages. Branch orientations did not apparently affect calcium content within clusters and levels in both leaves and flowers were similar until those in flowers stopped increasing between 'late green cluster' and 'first flower' (Figure 4.3.2.4b). Within clusters from the different ages of tree, the general pattern of increasing calcium concentration was the same as seen within the different ages of wood and orientations of branch (Figure 4.3.2.4c and d) but there were a few anomalies particularly within clusters from 2-year- old trees. In these clusters, calcium concentrations were higher at 'burst', 'mouse ear', 'pink bud' and 'full bloom' than they were in clusters from all other ages of tree.

Magnesium concentration varied throughout cluster development, declining slightly before 'mouse ear' before increasing up to 'late green cluster' and then declining slightly until 'full bloom'. Although concentrations within clusters from 2- and 3-year-old wood were very similar to each other, those within clusters from 1-year-old wood were different, being consistently lower within both leaf and flower tissue (Figure 4.3.2.5a). Clusters from horizontal and vertical wood had similar patterns of changing magnesium concentration and no differences were seen between them (Figure 4.3.2.5b). Within clusters from different ages of tree magnesium concentrations varied quite widely, most notable being the sharp decline in concentration within leaves and flowers of clusters from 6-year-old trees immediately before 'full bloom' (Figures 4.3.2.5c and d).

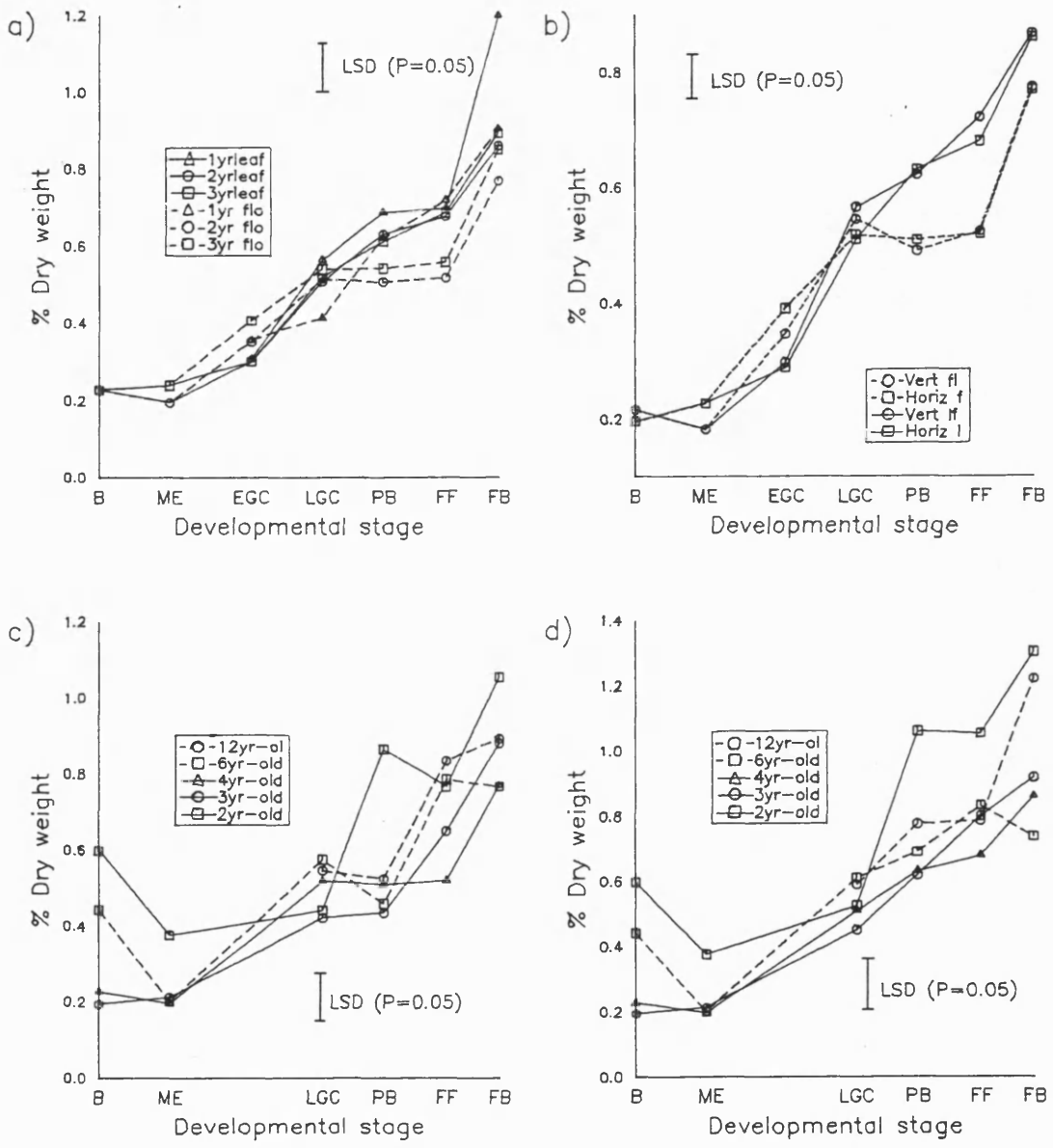
Within the clusters from the various wood ages and branch orientations, manganese concentrations generally increased between 'bud burst' and 'full bloom' but showed a marked decline between 'late green cluster' and 'pink bud' (Figures 4.3.2.6a and b). Concentrations were higher in leaves than flowers but virtually identical between clusters from the different wood ages and orientations. Between clusters from the different tree ages, although there



Figures 4.3.2.3a-d
Potassium concentration (% dry weight) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

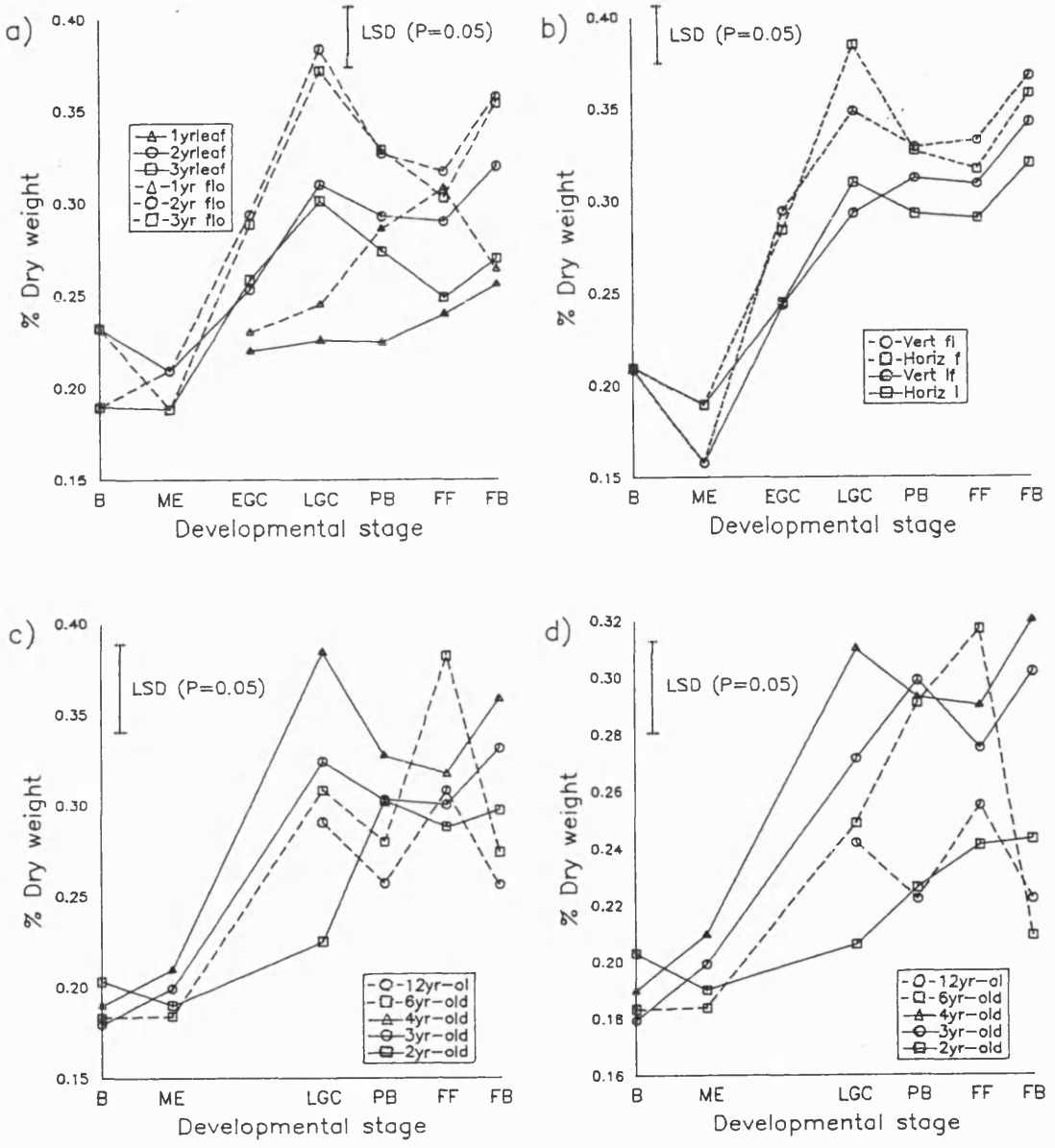
at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.2.4a-d
Calcium concentration (% dry weight) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

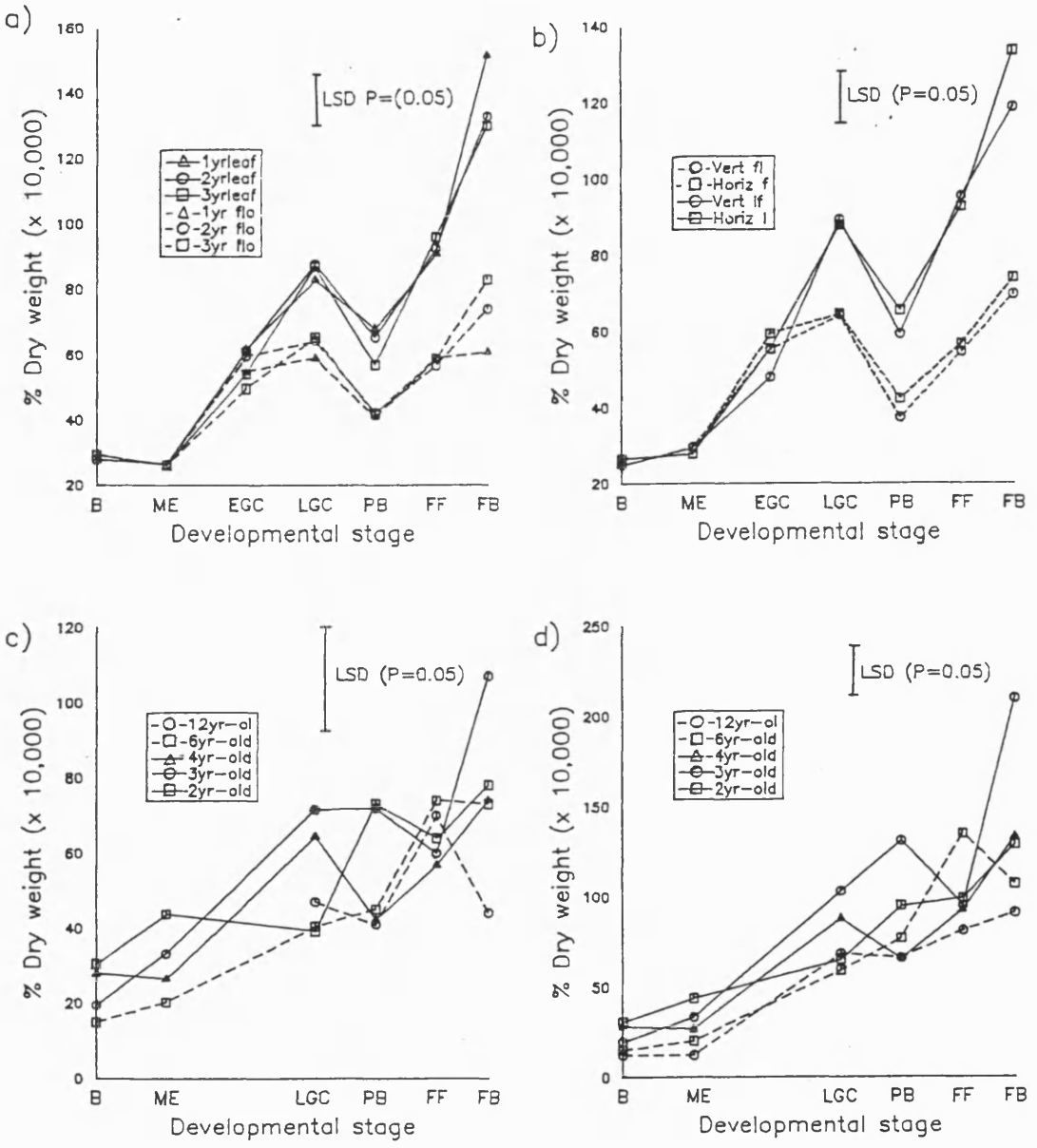
at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.2.5a-d
Magnesium concentration (% dry weight) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.2.6a-d
Manganese concentration (% Dry weight × 10^4) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.

was wide variability, concentrations generally increased slowly between 'break' and 'full bloom', rising from about $20x10^{-4}$ percent dry weight to $60x10^{-4}$ percent. Few major differences were seen between clusters from the different tree ages except for the sharp increase within the leaves and flowers of 3-year-old trees immediately before 'full bloom' (Figure 4.3.2.6c and d).

Zinc levels within leaves of clusters from the different wood ages and orientations of branch generally declined during cluster development and until 'pink bud' the pattern was very similar within flowers, after which concentration increased again (Figure 4.3.2.7b). Neither wood age nor branch orientation appeared to affect this. Within clusters from the various tree ages, zinc levels varied widely both between different developmental stages and also according to tree age (Figures 4.3.2.6c and d). Most notable was the high zinc concentration in 'mouse ear' buds from 2-year-old trees but although several significant differences were detected between clusters from different ages of tree at individual stages, these were highly variable over the whole period and no trends were apparent.

Sodium concentration in developing buds was markedly different for flowers and leaves (Figure 4.3.2.8a and b). Those in flowers remained relatively constant whilst those in leaves rose rapidly to a peak at 'late green cluster' and 'pink bud' before dropping sharply again. The pattern and degree of this change was almost identical within clusters from the different wood ages and branch orientations. Within clusters from the different tree ages, concentrations within flowers varied apparently widely, but in reality, over a very small range. Within leaves, levels followed a similar pattern to that already described except on 2-year-old trees where a sharp decrease occurred at 'pink bud'.

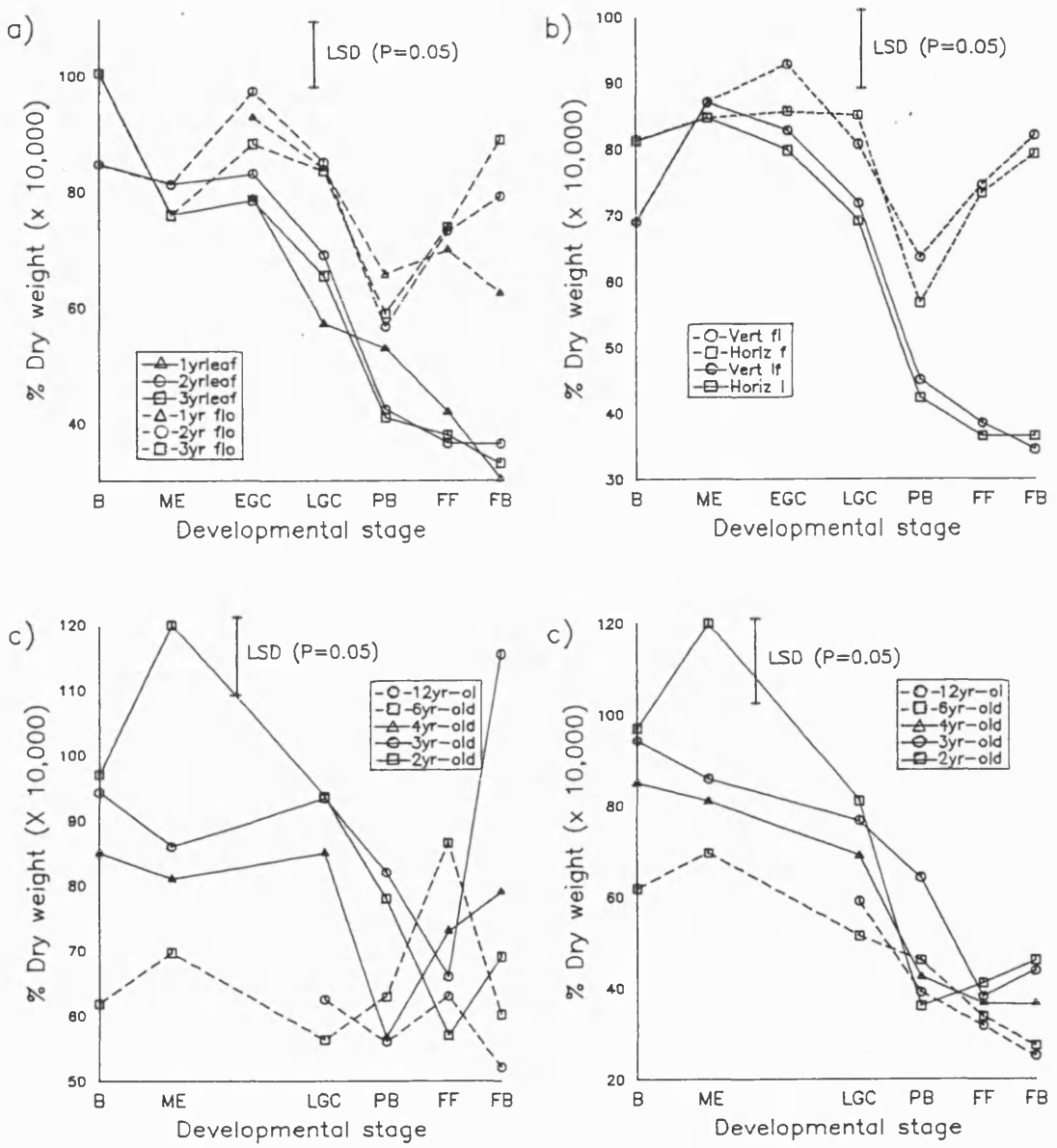
4.3.3 Chlorophyll content

In general, the concentration of chlorophylls a and b within developing cluster leaves increased steadily throughout leaf expansion. At 'green cluster', leaves had an average of 0.56 µg total chlorophyll per mg fresh weight of tissue; which rose to c 2.8µg/mg fresh weight at 'full bloom'. The ratio of chlorophyll a:b generally decreased as development progressed from 'green cluster' to 'full bloom' but no differences were seen in the pattern of this between clusters from different ages wood or orientations of branch (Table 4.3.3.1).

Within clusters from different ages of tree a similar pattern of increasing total chlorophyll and decreasing chlorophyll a:b ratio as development progressed was seen. No significant differences were found (Tables 4.3.3.2 and 4.3.3.3).

4.3.4 Leaf anatomy

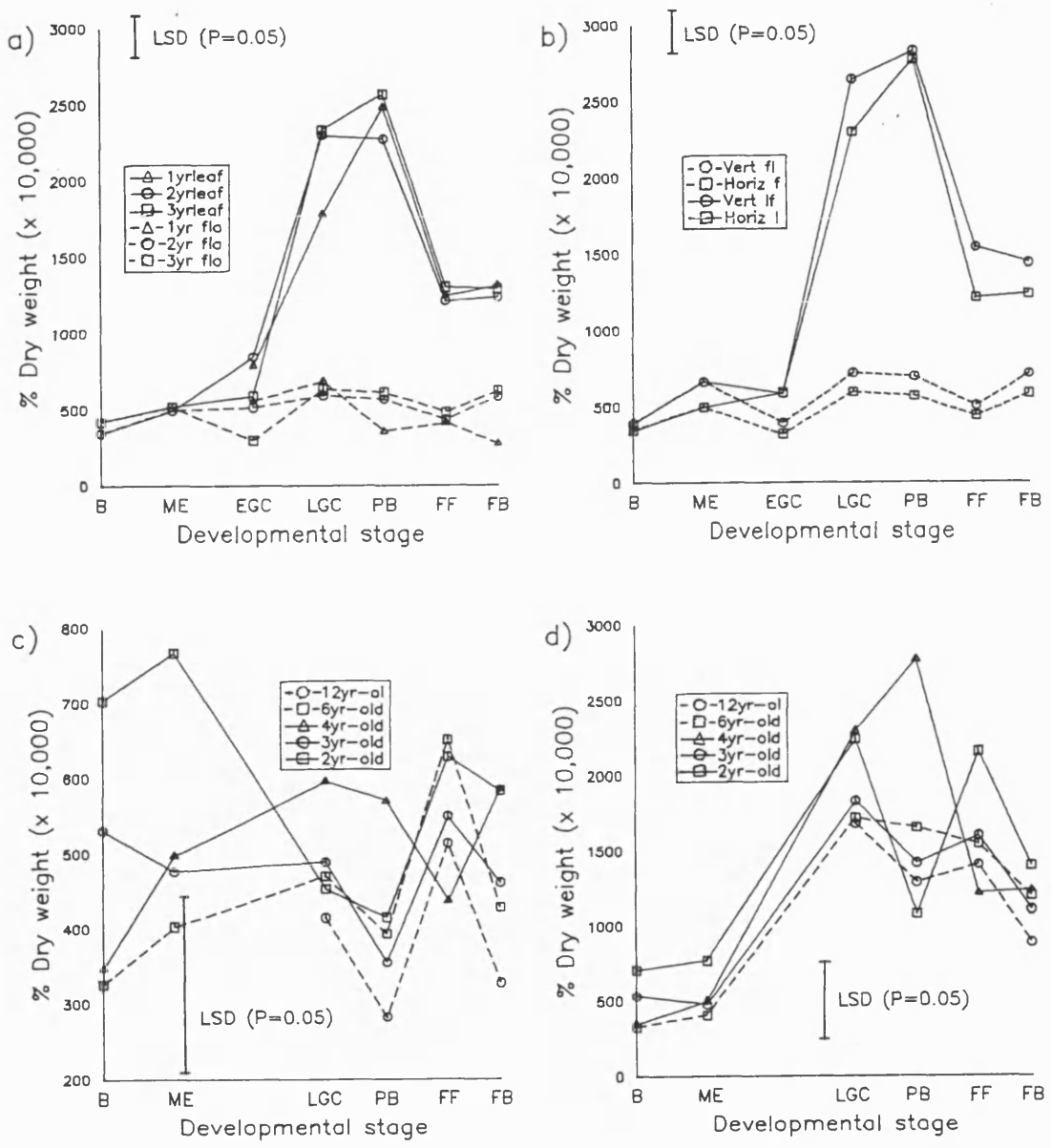
Apart from the 6-year-old trees, leaves from all ages of tree increased in thickness as clusters developed from 'green cluster' to 'pink bud', but then remained constant until 'full bloom' (Table 4.3.4.1). During this time leaf thickness on average increased from c 171µm to c 190µm



Figures 4.3.2.7a-d
Zinc concentration (% Dry weight × 10^4) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.



Figures 4.3.2.8a-d
Sodium concentration (% Dry weight × 10^4) within buds, flowers and leaves from various;

(a) ages of wood
(b) orientations of branch
(c) ages of tree (flowers)
(d) ages of tree (leaves)

at budburst (B), mouse ear (ME), early green cluster (EGC), late green cluster (LGC), pink bud (PB), first flower (FF) and full bloom (FB).
Values at B and ME represent weight of complete bud, i.e. leaves plus flowers.

Tables 4.3.3.1a-c Chlorophyll content (mg. chlorophyll/mg tissue fresh weight) of cluster leaves from various ages of tree. (a) chlorophyll a, (b) chlorophyll b and (c)ratio of chlorophyll a : chlorophyll b.

(a)

age of tree	developmental stage			
(years)	green cluster	pink bud	first flower	full bloom
2	0 · 530	1 · 106	1 · 290	1 · 613
3	0 · 420	1 · 410	1 · 734	2 · 093
4	0 · 493	1 · 200	1 · 586	2 · 101
6	0 · 402	1 · 141	1 · 120	2 · 021
12	0 · 307	1 · 184	1 · 271	1 · 907
S.E.D.		0 · 043		

(b)

age of tree	developmental stage			
(years)	green cluster	pink bud	first flower	full bloom
2	0 · 150	0 · 626	0 · 658	0 · 814
3	0 · 120	0 · 670	0 · 679	0 · 827
4	0 · 137	0 · 609	0 · 638	0 · 825
6	0 · 139	0 · 620	0 · 651	1 · 018
12	0 · 093	0 · 616	0 · 577	0 · 885
S.E.D.		0 · 017		

(c)

age of tree	developmental stage			
(years)	green cluster	pink bud	first flower	full bloom
2	3 · 5	1 · 8	2 · 0	2 · 0
3	3 · 5	2 · 1	2 · 6	2 · 5
4	3 · 6	2 · 0	2 · 5	2 · 5
6	2 · 9	1 · 8	1 · 7	2 · 0
12	3 · 9	1 · 9	2 · 2	2 · 2
S.E.D.		0 · 37		

Tables 4.3.3.2a-c Chlorophyll content (mg. chlorophyll/mg. tissue fresh weight) of cluster from horizontal and vertical branches. (a) chlorophyll a, (b) chlorophyll b and (c) ratio of chlorophyll a : chlorophyll b.

(a)

branch orientation	developmental stage			
	green cluster	pink bud	first flower	full bloom
horizontal	0 · 493	1 · 200	1 · 586	2 · 101
vertical	0 · 431	1 · 131	1 · 234	2 · 299
S.E.D.		0 · 052		

(b)

branch orientation	developmental stage			
	green cluster	pink bud	first flower	full bloom
horizontal	0 · 137	0 · 609	0 · 638	0 · 825
vertical	0 · 111	0 · 621	0 · 619	0 · 872
S.E.D.		0 · 021		

(c)

branch orientation	developmental stage			
	green cluster	pink bud	first flower	full bloom
horizontal	3 · 6	2 · 0	2 · 5	2 · 5
vertical	3 · 9	1 · 8	2 · 0	2 · 7
S.E.D.		0 · 032		

Tables 4.3.3.3a-c Chlorophyll content (mg. chlorophyll/mg. tissue fresh weight) of cluster leaves from 1-,2- and 3-year-old wood within a branch. (a) chloropyll a, (b) chlorophyll b and (c) ratio of chlorophyll a : chlorophyll b.

(a)

wood age (years)	developmental stage			
	green cluster	pink bud	first flower	full bloom
1	0 · 460	1 · 18	1 · 57	2 · 00
2	0 · 493	1 · 20	1 · 586	2 · 101
3	0 · 798	1 · 233	1 · 274	1 · 934
S.E.D.		0 · 048		

(b)

wood age (years)	developmental stage			
	green cluster	pink bud	first flower	full bloom
1	0 · 141	0 · 623	0 · 611	0 · 815
2	0 · 137	0 · 609	0 · 638	0 · 825
3	0 · 282	0 · 477	0 · 493	0 · 685
S.E.D.		0 · 26		

(c)

wood age (years)	developmental stage			
	green cluster	pink bud	first flower	full bloom
1	3 · 3	1 · 9	2 · 6	2 · 5
2	3 · 6	2 · 0	2 · 48	2 · 5
3	2 · 8	2 · 6	2 · 6	2 · 8
S.E.D.		0 · 33		

Tables 4.3.4.1a-c Thickness of leaves (μm) at three developmental stages on (a) various ages of tree, (b) horizontal and vertical branches and (c) different ages of wood within a branch.

(a)

age of tree (years)	developmental stage green cluster	pink bud	full bloom
2	180 · 1	199 · 1	204 · 6
3	160 · 6	203 · 4	206 · 4
4	174 · 4	197 · 3	195 · 2
6	176 · 4	177 · 1	169 · 5
12	166 · 0	180 · 6	177 · 2
S.E.D.		4 · 7	

(b)

branch orientation	developmental stage green cluster	pink bud	full bloom
horizontal	174 · 4	197 · 3	195 · 2
vertical	180 · 2	178 · 3	185 · 1
S.E.D.		4 · 8	

(c)

age of wood (years)	developmental stage green cluster	pink bud	full bloom
1	183 · 3	174 · 1	183 · 2
2	174 · 4	197 · 3	195 · 2
3	179 · 4	183 · 4	188 · 4
S.E.D.		4 · 6	

but leaves from 6-year-old trees appeared not to increase in thickness during this time, still having a depth of c 170 µm at 'full bloom'

At 'green cluster', leaves from 3-year-old trees were significantly thinner than those from 2-year-old trees but at 'pink bud' and 'full bloom' leaves from 2-, 3- and 4-year-old trees all had similar thickness and were significantly thicker than those from the 6 and 12-year-old trees.

Leaves from different orientations of branch showed similar increases in leaf thickness between 'green cluster' and 'pink bud' but no differences were observed between them (Table 4.3.4.1b).

Similarly no significant differences were seen between leaves from different ages of wood within a branch at any of the measurement times (Table 4.3.4.1c).

4.3.5 Uptake of $^{14}CO_2$

Results from the feeding and recovery of $^{14}CO_2$ to clusters throughout development were highly variable. Due to experimental constraints, use of only four replicates of each cluster position/developmental stage was possible. Results from these varied widely but whether these values reflect any real differences in CO_2 uptake or purely experimental error is hard to gauge. Although all possible attempts were made to control variables within these experiments, it is apparent that considerable variation did occur.

The $^{14}CO_2$ was generated within a feeding container by addition of excess hydrochloric acid to an aqueous solution of $Na_2{}^{14}CO_2$. The liquid was contained within a small vial attached to the inner surface of the feeding chamber, but on occasions, when the container was removed after feeding, liquid was found to have run down the inner wall of the container, presumably as a consequence of wind induced movement. If this was seen to have reached the cluster, the sample was discarded, but even so, on combustion some samples were found to have much higher levels of radioactivity within them than did others. Whether this indicates that they too had been unknowingly contaminated with the generating liquid is unknown. Occasionally, clusters or individual leaves would be broken as a result of damage during the feeding process and in each case loss of such samples meant replication being reduced. When combusted, some samples had far lower ^{14}C levels than did the majority. For example, in one pink bud sample from a 6-year-old tree flowers had 471 counts per mg (cpmg), the average of the other three replicates being 4,258 ± 818. In this case the level of counts within the leaf tissue was also low compared to the other replicates (1,857 cpmg compared to 23,475 ± 3,134) indicating that either the $^{14}CO_2$ had not been generated properly, that the generated gas had subsequently escaped, or that attachment of the feeding chamber had rapidly killed the cluster. Chambers were inspected before every feeding and repairs were made where necessary. Care was taken to ensure complete mixing of the $Na_2{}^{14}CO_2$ and HCl but

obviously something was going wrong somewhere. Whatever the reason, results overall were highly variable and it is doubtful whether anything conclusive can be drawn from them.

In general, feeding of flower clusters from which spur leaves had previously been removed resulted in a higher concentration of ^{14}C within them than within the flowers of fed clusters which still had their leaves attached. Of the clusters with leaves remaining, no differences could be seen between those from horizontal and vertical branches (Table 4.3.5.1a). Where leaves had been removed prior to feeding, although not significant, flower buds from vertical branches had consistently higher levels of ^{14}C within them than did those from horizontal branches.

Within clusters from the various tree ages results were, if anything, slightly less variable than they had been for clusters within the same tree (Table 5.3.5.2). Generally when flowers had been fed with spur leaves attached they had taken up about 1000 cpmg (one exception was the 'pink bud' sample on 2-year-old trees where 4,473 cpmg had been absorbed). In clusters fed after spur leaf removal (Table 5.3.5.2b) uptake was around 4,250 cpmg - (but again the 'pink bud' sample from 2-year-old trees was much higher at 9,886 cpmg). However the mean error on this sample was very large due to one cluster having 18,730 cpmg - omitting this result from the calculation gave a mean of 4,195 cpmg.

Overall, the experiment did not show any conclusive differences in ^{14}Carbon uptake or accumulation either between clusters at different developmental stages, clusters on different branch orientations or those on different ages of tree, in addition it did not give any information regarding how much ^{14}C the cluster leaves were contributing to the developing flowers within each age of tree.

4.4 Discussion

Overall, the attempt to relate any morphological or physiological differences between flowers borne on various ages of wood, orientations of branch or ages of tree to their different setting abilities described in this section and illustrated in Chapter 5, gave inconclusive results.

However, buds on both the youngest trees and the youngest wood developed later than did those on older trees and older wood. Delayed bud development has been observed before in circumstances associated with poor fruit set. Buszard (1983) found that clusters from previously defruited trees developed ahead of those from heavily cropped trees and that the former set fruit much more readily than did the latter, and similarly Roberts (1947) observed late blossoming in 'Delicious' to be associated with weak spurs.

This delayed flowering may reflect either a reduced availability of stored reserves necessary to sustain initial growth and/or a less advanced stage of development reached by the buds prior to dormancy the year before.

Table 4.3.5.1. Uptake of $^{14}CO_2$ (d.p.m./mg. dry weight) and (S.E.M.) of flower clusters on horizontal and vertical wood. Clusters, either with or without spur leaves were fed $^{14}CO_2$ for 1 hour, then harvested 24 hours later.

	with spur leaves		without spur leaves	
branch orientation	horizontal	vertical	horizontal	vertical
developmental stage				
green cluster	1,895	3,019	4,800	4,857
	(1,000)	(999)	(2,439)	(2,337)
pink buds	3,520	3,231	2,743	5,029
	(896)	(921)	(1438)	(2344)
first flower	903	607	2,937	4,266
	(202)	(167)	(1012)	(1574)
full bloom	899	1,194	2,543	3,982
	(328)	(377)	(1,521)	(1,429)

Tables 4.3.5.2a + b Uptake of $^{14}CO_2$ (d.p.m./mg. dry weight) and (S.E.M.) of flower clusters from various ages of tree. Clusters, either with (a), or without (b) spur leaves were fed $^{14}CO_2$ for 1 hour, then harvested 24 hours later.

(a) = with spur leaves

developmental	age of tree (years)			
stage	2	4	6	12
green cluster	1,168	1,066	934	1,131
	(439)	(253)	(188)	(354)
pink bud	4,473	1,237	809	3,312
	(380)	(285)	(306)	(1,109)
first flower	952	1,046	777	1,965
	(53)	(244)	(328)	(982)
full bloom	990	896	859	535
	(105)	(254)	(396)	(177)

(b) = without spur leaves

developmental	age of tree (years)			
stage	2	4	6	12
green cluster	3,046	3,676	2,359	3,242
	(384)	(358)	(364)	(290)
pink bud	9,886	4.002	5,694	6,446
	(3,332)	(687)	(2,661)	(1,960)
first flower	4,863	4,959	3,971	3,892
	(973)	(1320)	(1985)	(655)
full bloom	5,219	3,672	4,396	4,542
	(1,068)	(689)	(843)	(998)

Flower initiation and initial development occurs during the summer prior to flowering. Therefore conditions operating at this time are capable of influencing the stage of development reached by the bud before dormancy, and also the level of stored reserves within it. Timing of flower initiation may be important in this and it has been reported to occur later on 1-year-old wood compared to older wood (Zeller 1960) and also on vigorously growing (young) trees compared to less vigorous older ones (Auchter and Schrader 1923, Luckwill 1970). The length of time between floral initiation and flowering the following year has been shown by Abbott (1970) to have a great effect on the morphology and fruit setting ability of the flowers subsequently produced. When flower initiation occurs late in the season and the flower clusters produced are physiologically 'young', fruit set is very poor. By contrast, when floral initiation occurs early, and physiologically 'old' clusters are formed, fruit set is much better. Even if initiation occurs simultaneously on all ages of wood (Luckwill and Silva 1979) or ages of tree, the level of reserves built up may still vary according to individual circumstances. It is generally agreed that apple buds accumulate reserves during the late part of the season when shoot growth rate is declining but leaves still remain functional (Williams 1973, Williams *et al.* 1980). Preistley (1964) emphasised the importance of autumn foliage in influencing the carbohydrate status of the apple tree as a whole and Davis (1957) showed that currently produced photosynthates are necessary for floral initiation. Southwick *et al.* (1967) found that reducing the leaf area of non-bearing spurs to two leaves at the time of floral initiation significantly reduced the degree of flowering and fruit set.

Thus flowers from these two situations studied (young trees and young wood) may perhaps either be initiated late, and/or suffer from a lack of nutritional reserves due to a smaller leaf area from which to draw resources.

Work by Palmer (1988) showed that in the first orchard year, because young trees have very few spur leaves, leaf area index (LAI) started to accumulate only when extension growth was underway and did not reach a maximum until early October. Consequently even if floral initiation did occur early in the season there would be little assimilate available for primordial growth. It was seen that on the same trees in the following year, more spur leaves were available initially, and thus LAI built up more rapidly and reached a maximum by late July. The next year (i.e. the third year in the ground) LAI built up even more rapidly and reached a peak in June; any flowers initiated from June onwards would therefore have had the maximum leaf area available to provide assimilate for growth and development. This may indicate a very plausible reason for the late development of the buds on the young trees and also their smaller size throughout. Similar constraints would operate on flowers initiated on 1-year- old wood where by its very nature leaves are only present later in the season and because leaves are only produced as a consequence of more shoot growth, LAI may never reach a high level. Leaf area therefore is never so profuse as on older wood.

Both Hill-Cottingham and Williams (1967) and Miller (1988) have shown that flower 'quality' is not just an expression of differences inherent in buds before dormancy, but can be altered by either autumn or spring treatments. However, since in this study conditions during dormancy and spring (in terms of temperature and fertilisation regime) were the same for all ages of wood and all ages of tree, then it might be suggested that the factors operating either before dormancy and/or after bud 'break' were of more importance here.

It is interesting that leaf area was consistently lower in clusters from both 1-year-old wood and 2-year-old trees compared to those from older wood and trees. However, leaf size is not always positively related to flower quality. Abbott (1970) found that clusters which had only a short time between flower initiation and subsequent flowering (i.e. 'young' clusters) had larger leaves than 'old' clusters although the latter set fruit more readily, and similarly Buszard (1983) found no difference in leaf area between flowers of different 'qualities' and setting abilities. However, some people have associated small leaves with poor 'quality' clusters (Abbott 1971), and leaf removal during flowering does reduce fruit set (Ferree and Palmer 1982 and Chapter 5).

Within this experiment it was seen that leaves of clusters on 3-year-old trees were of equivalent area to those on 6-year-old trees, and larger than those on 4- and 12-year-old trees even though fruit set would generally be expected to be poorer on the clusters from the young trees. It is shown in Chapter 5 that fruit set was indeed poorer on the 3-year-old trees compared to the 6- and 12-year-old ones, and the combination of these two results may suggest that leaf area is not a crucial factor in fruit set. However, the greater reduction of leaf area within clusters on the 1-year-old wood and 2-year-old trees may have been sufficient to cause a problem.

Of interest are the mineral concentrations within the leaf and flower tissues. Although when the varying concentrations of individual elements are examined seperately, no major differences stand out, clusters from 2-year-old trees and 1-year-old wood had significantly higher concentrations of two minerals - calcium and potassium - at the later developmental stages. However it is perhaps unlikely that flower 'quality' is being reduced by the raised concentrations. Potassium is involved in stomatal movements and the activation of many enzymes, and calcium is a requirement for new middle lamella production in cell plates. Toxic excesses of either are unusual in plants under normal nutritional conditions. It is possible though, that these concentrations might have arisen due to a lack of dilution by carbohydrate. If these clusters were receiving less carbohydrate than were those from older wood and trees, but mineral content continued to increase, then concentrations would inevitably rise. If this was the case then you might expect all, or at least more, minerals to show this increased concentration. No other mineral concentration stood out as being markedly different to those within clusters from the older trees and wood, and all were above levels normally associated with deficiency. One

point that may be worth mentioning is the wide range of concentrations found at any one sampling time within the clusters from the various tree ages. When clusters came from within individual trees of the same age and the same orchard, (i.e. when wood age or branch orientation was being examined), there was generally little difference between the mean values obtained for each examined situation. This would suggest that the wide range of means found when comparing separate orchards, reflected real differences between the clusters there rather than just high levels of variation between all individual clusters. However, whether these are due to tree age rather than simply to orchard differences is unknown.

It is perhaps not surprising that chlorophyll concentration was not affected by cluster position. The literature indicates that in the absence of nutrient deficiency, and prior to degredation of chlorophyll in the autumn, variation in light intensity affecting the leaves is the main cause of altered chlorophyll concentration (Salisbury and Ross 1978). Jackson and Beakbane (1970) showed that when mature apple trees were grown under a range of shade conditions leaf thickness was linearly related to light intensity. Therefore because no differences in leaf thickness dependent on cluster situation were observed in this present experiment, it might suggest that no strong shading effects were operating and consequently that chlorophyll levels would not have been affected either.

Thus, few conclusions concerning the physiological reasons underlying variations in flower 'quality' can be drawn from this section of the work. It cannot be concluded that any specific differences in mineral content, chlorophyll content, leaf thickness or the levels of minerals within the clusters have any association or causal role in determining the 'quality' and setting ability of a flower. All that can be stated is that within the situations where severely reduced fruit set is often seen (axillary flowers and flowers on very young trees), cluster development was delayed, and the subsequent leaves and flowers were consistently smaller than in flowers which would be expected to set fruit more successfully.

Chapter 5. Female fertility and effective pollination period as affected by tree age, wood age, branch angle and shoot tipping.

5.1 Introduction.

Female fertility has been described as 'the capacity of a flower to develop into a fruitlet if pollinated with the right pollen at the right time' (Williams 1970a). The length of time during which such pollinations result in fruitlet formation is known as the 'effective pollination period' (EPP) (Williams 1965) and is known to be a function of pollen tube growth rate and ovule longevity.

Effectively it is the length of time that ovules remain viable minus the length of time taken for pollen to germinate on the stigma and grow through the style to reach them (Williams 1970). Thus in order to achieve fertilisation and fruit set several criteria must be met.

Firstly the stigma must present a receptive surface to allow pollen adhesion and also to provide an environment suitable for germination. At anthesis stigmas usually bear numerous papillae whose secretions nourish the developing pollen, and in both apple (Braun and Stosser 1985, Miller 1988) and Pear (Herrero 1983) the physical condition of this surface has been shown to change as time after anthesis increases. These changes may well alter stigma receptivity to pollen, and therefore, also affect EPP. Although Braun and Stosser (1985) found little evidence to support this, the question was investigated and is reported and discussed in Chapter 6.

Secondly, the rate at which the pollen tube grows through the style must be sufficient to ensure that the tube reaches the ovary before the embryo sac degenerates. The length of time taken by a pollen tube to travel through the style is not necessarily dependent on the distance travelled. In crocus, where the style is 6-10 mm. long, only one to three days are required, but in meadow saffron although the style is about the same length, the time between pollination and fertilisation is around six months (Esau 1961). Within apple, pollen tube growth rate is highly dependent on temperature (Child 1967), increasing almost linearly with it. At 7°C pollen tubes require approximately 10 days to reach the style base but at 15°C only 2 days are required (Williams 1970b). This might suggest that as temperature during the flowering period increases, fruit set will also increase due to higher rates of pollen tube growth. However, pollen tube growth is not the only component of fruit set to be affected by temperature; ovule longevity is also thermo-sensitive, with high temperatures being detrimental. Vasilakakis and Porlingis (1985) showed that the EPP of flowers on branches contained within cheesecloth was longer than that of flowers on branches contained in cellophane (where the temperature was 7°C higher) and Williams (1970) reported that ovule senescence was accelerated by high temperatures.

The third factor affecting EPP is that at the time when the pollen tube reaches the ovaries they must be fully developed, still meristematically active and with the correct spatial arrangement of nuclei within their embryo sacs. Embryo sacs often only reach full maturity at or after anthesis (Williams 1970) and they remain in this condition for only a finite time after which they lose their internal organisation and consequently, the ability to be fertilised and commence seed development (Dorsey 1929, Williams1965). As many seperate experiments have shown, the length of time ovules remain viable can be affected by many factors including variety (Williams 1966), ploidy (Howlett 1938, Williams 1970), tree nutritional or vigour status (Dorsey 1929) or even weather (Williams 1970). Dorsey (1929), in the first detailed investigation into the relationship between fruit set and ovular condition, found a strong relationship between the condition of the embryo sac at anthesis, and the level of fruit set obtained. He also noted that the ovules of 'Delicious', a variety widely recognised to set fruit badly (Howlett 1928, Dennis 1979), had a lower proportion of correctly developed embryo sacs than did those of varieties which set more readily.

Williams (1970) stated that ovules of triploid varieties remained viable for a longer time period than did those of diploid varieties, this being attributed to the greater amount of meristematic activity within ovules of the former and the consequent extended ovule longevity.

In another experiment, Williams (1965) found that nitrogen application during the summer increased the time period during which ovules remained meristematically active the following year and by doing so, increased the length of EPP from 7-9 days to 12-13 days.

Similarly, Buszard (1983) found that flowers from previously defruited trees had longer EPPs than did those from heavily cropped trees presumably due to the increased nutrition available to them.

That temperature can have a direct effect on ovular condition and therefore EPP was shown by Miller (1988) where trees subjected to warm spring temperatures were found to have flowers with a shorter ovule life and EPP than did those from trees subjected to cool spring temperatures.

Thus the EPP of a flower can be determined by either the length of time that a stigma remains receptive, the length of time taken for pollen tubes to reach the ovary or ultimately, the length of time that ovules remain viable.

Although several workers have shown that EPP can be variable depending on growth and climatic conditions, little is known about how this aspect of fruit production is affected either by the age of tree upon which a flower was borne, or its position within the tree (i.e. the age of wood or orientation of branch upon which it was borne). Similarly, although several cultural treatments used to promote fruit production have been reported to increase fruit set (e.g. scoring (Southwick *et al.* 1967, Vienbrants 1972), removing shoot tips (Quinlan and Preston 1971)), the effects of others (e.g. changing branch angle from upright to horizontal) are less predict-

able. There are many references advocating the training of branches to a horizontal position to encourage fruit production (Goldschmidt and Delap 1950, Dennis 1979, Greene 1981) but experimental investigation into this technique has produced variable results. Often only flower production is recorded (Wareing and Nasr 1958, Tromp 1968, 1970, 1972) and the response of this has varied from no effect (Dermine and Monin 1960, Jonkers 1962, Mika 1969) to substantial improvement (Tromp 1968, 1972, 1973, 1987). However, even if a horizontal branch orientation does not stimulate flower production, it can still potentially increase yield if fruit set is increased instead. Because many studies have recorded only flower production (Tromp 1968, 1972, 1987) or final yield (Preston 1974), there is little information regarding this.

Consequently the aim of the experiments described in this chapter was to investigate the extent to which female fertility and EPP might be affected by the following factors:

a) age of wood and orientation of branches within a tree,

b) age of tree,

c) horizontal or vertical training of branches either during flower initiation (August) or immediately prior to flowering (April),

d) removal of shoot tips from horizontal and vertical branches either during flower initiation (August) or immediately prior to flowering (April).

5.2 General materials and methods.

The female fertility of flowers on various ages of tree, ages of wood, orientations of branch and also on tipped and untipped branches was assessed within separate experiments. The trees used were as described in Section 2.2. Because the timing of flower development sometimes varied dependent on the situation in which the cluster was borne, care was taken to ensure that all experiments started with flowers at the same stage of development independent of the date at which this was reached. Consequently, floral development within all the locations under examination (i.e. ages of tree, ages of wood, angles of branch) was monitored and when, approximately 20% of flowers had opened (mostly king flowers) in a particular location, clusters with lateral flowers at the 'late balloon' stage were selected. The king flower was removed and the cluster thinned to four or five similar laterals prior to being enclosed in a waterproof glassine paper bag. Although Goldwin and Schwabe (1975) found fruit with seeds on Cox trees enclosed in muslin cages, and Williams and Maier (1977) found that selfing can occur in Cox flowers at high temperatures, generally Cox flowers are considered to be highly self-incompatible (Spiegel-Roy and Alston 1982), and therefore due to time constraints, flowers for female fertility studies were not usually emasculated.

Starting on the day of bagging, and then proceeding every 2 days for the next 6 to 10 days, one or two clusters per tree (20-25 clusters per situation) were hand pollinated using a mixture of Miller's Seedling and Golden Delicious pollen. Where clusters had been thinned to five lateral flowers, one of these was removed for histological work prior to pollination of the re-

mainder. Bags were removed 14 days after bagging, and the number of fruitlets set from the hand pollinated flowers were counted 21 days after pollination.

For pollen collection, anthers were combed off flowers, dried in an incubator at 30°C until dehisced, then placed in a glass vial and shaken vigorously to release the pollen. Pollen was stored in a desiccator at -20°C and aliquots were removed when required. Pollen viability after storage at -20°C was tested by using the (10%) sucrose hanging drop technique. Germination was always in the range of 60-80%.

5.3 Experimental materials and methods

5.3.1 Experiment 1 : Assessment of female fertility and effective pollination period of flowers borne on different ages of wood and orientations of branch within a tree.

Six flower clusters were selected for uniformity at the 'late balloon' stage on both horizontally and vertically growing wood of 1-, 2- and 3-years age on each of twenty five 3-year-old trees. Blossom on 2- and 3-year-old wood reached this stage on the same date (May 8th), those on 1-year-old wood did so approximately five days later. Clusters were thinned to four laterals, bagged and pollinated as described in Section 5.2. Starting on the day of bagging and continuing at 1 or 2 day intervals for the following 9 days one bagged cluster per branch orientation/age combination on each tree was pollinated. Bags were removed 14 days after bagging and fruitlets counted 21 days after pollination.

5.3.2 Experiment 2: Assessment of female fertility and effective pollination period of flowers borne on different ages of tree.

In 1985, the effective pollination period (EPP) of flowers borne on 2-, 3-, 4-, 6- and 12-year-old trees was assessed; in 1986 2-, 3-, 5- and 7-year-old trees were studied in a similar way.

Clusters at the 'late balloon' stage, borne on 2-year-old horizontal wood were selected for uniformity when approximately 20% of the other flowers on the same trees had opened. The king flower was removed and clusters were thinned to five lateral flowers.

In 1985, the majority of flowers on the 3-, 4-, 6- and 12-year-old trees reached this stage simultaneously (12th May). Those on 2-year-old trees were delayed by approximately 3 days. In 1986 clusters on all ages of tree developed simultaneously, all pollinations therefore commenced on the same date (19th May).

Starting on the day of bagging and proceeding every 2 days for the following 8 (1986), or 10 (1985) days, one cluster per 2- and 3-year-old tree and two clusters on all other tree ages (a total of 24 clusters per tree age), were assessed. On each cluster one lateral flower was removed for histological examinations, and the remainder were hand pollinated. Bags were removed 14 days after bagging. The number of fruitlets set by these pollinations were counted approximately 7 days later.

In supplement, in 1985 an experiment was run to assess how defoliation affected the relative development and setting ability of clusters on each age of tree. As clusters approached the 'pink bud' stage, thirty pairs were selected within 2-year-old horizontal wood on each age of tree and within 2-year-old vertical wood within 4-year-old trees, thinned to five similar laterals and labelled. At 'pink bud' one cluster from each pair was defoliated. At anthesis the largest lateral on each cluster was removed and its receptacle diameter and pedicel length measured. The remaining flowers were hand pollinated, fruit set being assessed 21 days later.

5.3.3 Experiment 3: Determination of the time period during which branch orientation exerts an influence over the fruit setting ability of flowers.

With fruit buds at the 'mouse ear' stage (April 10th), six branch units comprising a 2-year-old section of wood bearing several fruit buds, on each of twelve 6-year-old Cox/MM106 trees, were changed from a predominately vertical orientation to a horizontal one. Six similar branches were left unchanged. Simultaneously six horizontal branch units were manipulated to a vertical orientation, whilst six similar ones remained horizontal. Thus within each of the 12 trees four situations were compared,

(i) Branch units vertical during flower initiation, horizontal at blossoming,

(ii) Branch units vertical during both flower initiation and blossoming,

(iii) Branch units horizontal during flower initiation, vertical at blossoming,

(iv) Branch units horizontal during both flower initiation and blossoming.

On each tree, two clusters per branch unit were selected for uniformity at the 'late balloon' stage, thinned to four lateral flowers then enclosed in glassine bags. Starting on the day of bagging and continuing at 2-day intervals for 10 days two clusters per treatment per tree were pollinated. Bags were removed 14 days after bagging and fruitlets set were counted 21 days after pollination.

5.3.4 Experiment 4: Determination of shoot tip influence on the quality of flowers borne on different orientations of wood, and assessment of the time period during which this influence operates.

In July 1985, horizontal and vertical branches of 4-year-old trees were assigned one of three treatments as follows,

(i) All subtending extension shoot tips removed on August 1st 1985

(ii) All subtending extension shoot tips removed on April 10th 1986

(iii) Control - no shoot tip removal

Each branch unit used consisted of a 2-year-old section of wood bearing several nodes. Three such units were used for each treatment/orientation combination within each of twelve trees.

Shoot growth from each branch unit was measured in December 1985 and the number of fruit buds produced counted in April 1986. On each branch unit four clusters (i.e. 72 clus-

ters/tree) were selected for uniformity at the 'late balloon' stage. These were thinned to four lateral flowers and enclosed in glassine bags.

Starting on the day of bagging and continuing at 2 day intervals for 10 days, two clusters per orientation/treatment on each tree were hand pollinated. Bags were removed 14 days after bagging and the fruitlets counted 21 days after pollination.

5.4 Results

5.4.1 Experiment 1: Assessment of female fertility and effective pollination period in flowers borne on different ages of wood and orientations of branch within a tree.

The age and orientation of wood on which flowers were borne significantly affected the proportion of these which set fruit.

Considering age of wood first, averaged over the whole pollination period, flowers on 2-year-old wood set the most effectively, 38.5% of pollinations resulting in fruitlet formation (Table 5.4.1.1). Flowers on 3-year-old wood set at a similar level with 34.7% of the flowers pollinated during the experimental period setting fruit. Flowers on 1-year-old wood set fruit least successfully with only 15% of pollinations producing fruitlets.

Examination of the percentage fruit set arising from individual pollination dates, within the different ages of wood, showed that of the flowers borne on 2-year-old wood, the highest percentage fruit set (62%) was obtained from the first date of pollination (i.e. 'late balloon'). After this time percentage fruit set declined throughout the flowering period, such that only 15.2% of flowers pollinated 9 days later set fruit (Figure 5.4.1.1).

The percentage set of flowers borne on 3- and 1-year-old wood followed a similar pattern with time of pollination. That of flowers on 3-year-old wood decreased from 57% to 12.9%, those on 1-year-old wood from 29% to 3%.

Although consistently higher for individual pollination dates, the percentage set achieved by flowers on 2-year-old wood was not always significantly greater than that of flowers on 3-year-old wood, but was when taken as an average over all pollinations (Table 5.4.1.1). On all pollination dates the percentage fruit set obtained from flowers on axillary wood was significantly lower than that obtained from those on either 2- or 3-year-old wood.

Examination of the influence of branch orientation on fruit set indicated that averaged over all other variables (i.e. ages of wood and pollination dates), flowers on horizontal branches set significantly more fruit (32.4%) than did those on vertical branches (24.2%) (Table 5.4.1.1).

This difference was consistent, though not always significant for all pollination dates (Figure 5.4.1.2). As pollination was increasingly delayed, a parallel pattern of decreasing percentage fruit set of flowers on both branch orientations was observed.

Table 5.4.1.1. Percentage fruit set (and standard error of the mean) of flowers on various ages of wood within horizontal and vertical branches. Pollinations were carried out at five times between late balloon and nine days later. Data was analysed using analysis of deviance with a binomial model.

age of wood (years)	branch orientation horizontal	vertical	age mean
1	17 · 8	12 · 2	15 · 0
	(1 · 15)	(0 · 92)	(0 · 93)
2	43 · 4	33 · 4	38 · 5
	(1 · 76)	(1 · 67)	(1 · 49)
3	39 · 4	29 · 8	34 · 7
	(1 · 67)	(1 · 53)	(1 · 39)
orientation mean	32 · 4	24 · 2	
	(0 · 99)	(0 · 94)	

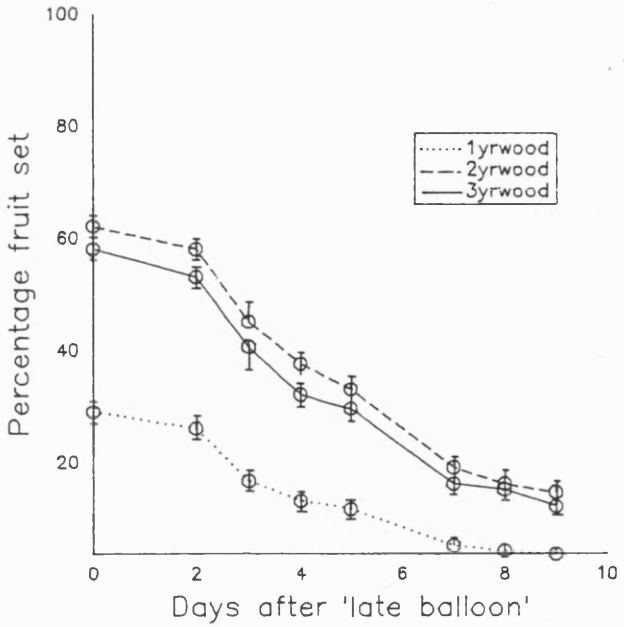


Figure 5.4.1.1.
Percentage fruit set of flowers borne on 1-, 2- and 3-year-old wood pollinated at increasing lengths of time after 'late balloon'. Bars indicate S.E.M.

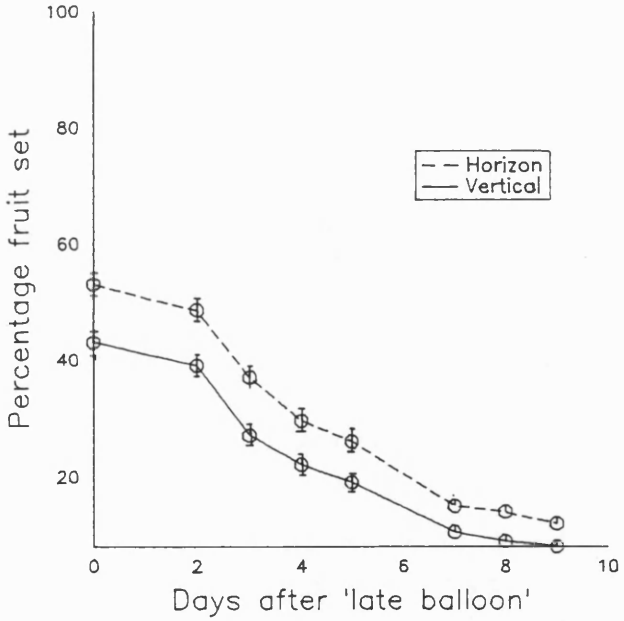


Figure 5.4.1.2.
Percentage fruit set of flowers borne on horizontal and vertical branches pollinated at increasing lengths of time after 'late balloon'. Bars indicate S.E.M.

Examination of branch orientation effects within each age of wood showed that when all pollinations were considered together, flowers on horizontal branches of each age of wood set more fruit per pollination than did those on vertical branches (Table 5.4.1.1).

On 1-year-old wood, flowers on horizontal branches set almost 50% more successfully (17.8% of pollinations resulting in fruit set) than did those on vertical branches (12.2% of pollinations producing fruit). On the 2- and 3-year-old wood, flowers on horizontal branches set approximately 30% more fruits per hundred clusters than did those on vertical branches.

Percentage fruit set obtained from flowers on 1-, 2- and 3-year- old wood within horizontal and vertical branches are shown in figures 5.4.1.3a - c. From these it can be seen that on all ages of wood, and on each date of pollination, flowers on horizontal branches consistently set more fruit than did those on vertical branches.

Also, within both branch orientations, flowers on 2-year-old wood set consistently, but not significantly, better than did those on 3-year-old wood and flowers on both of these wood ages set significantly more fruit than did those on 1-year-old wood (Figures 5.4.1.4 a and b).

5.4.2 Experiment 2: Assessment of female fertility and effective pollination period of flowers borne on different ages of tree.

In both 1985 and 1986 the percentage of flowers which set fruits decreased as pollination was progressively delayed during the flowering period (Figures 5.4.2.1 a and b).

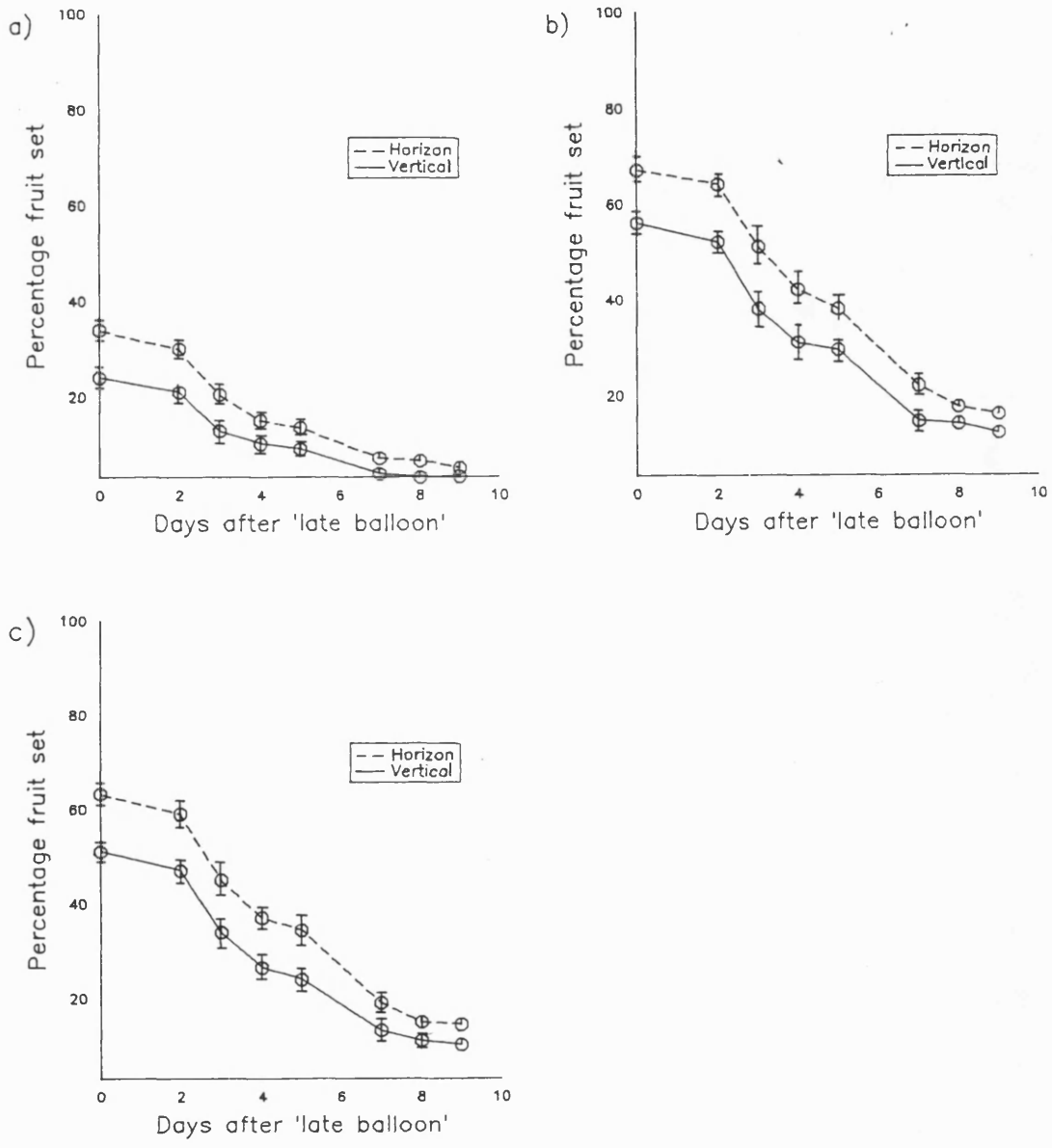
The pattern of this decrease varied both between years and ages of tree.

On all ages of tree in 1985, the percentage of flowers setting fruit from pollinations on either the 1st, 2nd or 3rd date was similar. Percentage fruit set resulting from subsequent pollinations decreased dramatically as time after 'late balloon' increased.

In 1986 however, within all ages of tree, the highest values of percentage fruit set were obtained when pollination occurred at the 'late balloon' stage. As pollination was increasingly delayed beyond this time percentage fruit set declined markedly. In both years of study, the age of tree upon which a flower was borne significantly influenced its ability to set fruit.

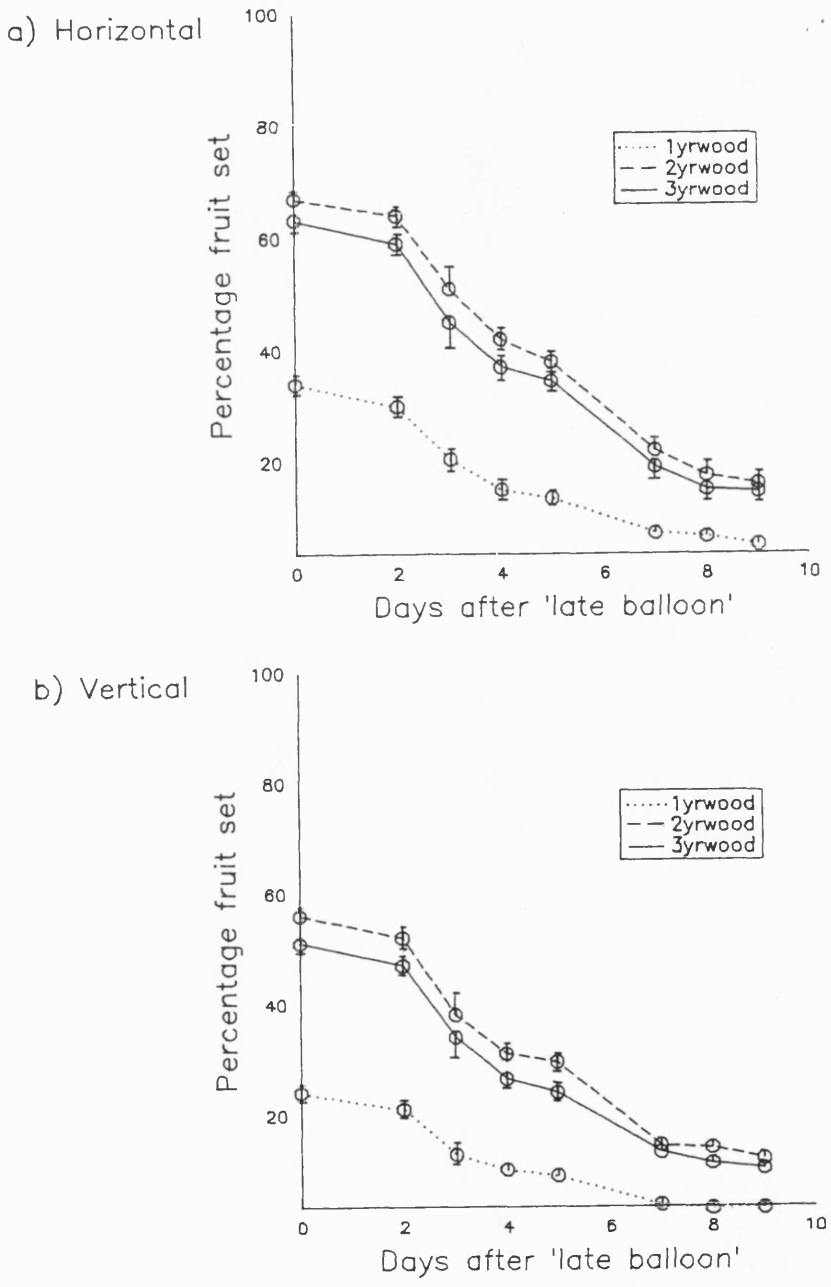
In 1985, within the 3- and 4-year-old trees, percentage fruit set arising from each date of pollination was very similar. Pollinations at 'late balloon' produced an initial set of 56% and 57% respectively, rising to 60% and 62% when pollination occurred four days later. Set then decreased sharply to 3.8% and 3.6% following pollination on day 10.

Fruit set on the 6-year-old trees was significantly higher than on the 2-, 3- and 4-year-old trees on all pollination dates. After pollination at 'late balloon', set was 73%, rising to 77% from pollination 4 days later and then declining to 7.6% when pollination was delayed for a further 6 days. The 12-year-old trees showed an intermediate response to pollination. On these, 65% of flowers pollinated at 'late balloon' set fruit, whereas pollination 4 days later gave 72% fruit set. This then declined to 5.8% when pollination occurred 10 days after 'late balloon'.



Figures 5.4.1.3a-c
Percentage fruit set of flowers borne on various ages of wood within horizontal and vertical branches pollinated at increasing lengths of time after 'late balloon'. Bars indicate S.E.M.

(a) 1-year-old wood
(b) 2-year-old wood
(c) 3-year-old wood

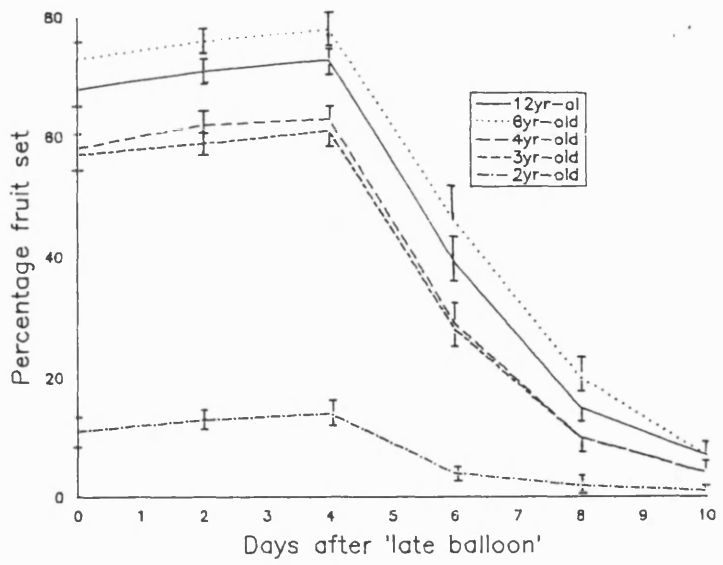


Figures 5.4.1.4a + b
Percentage fruit set of flowers borne on various ages of wood within;

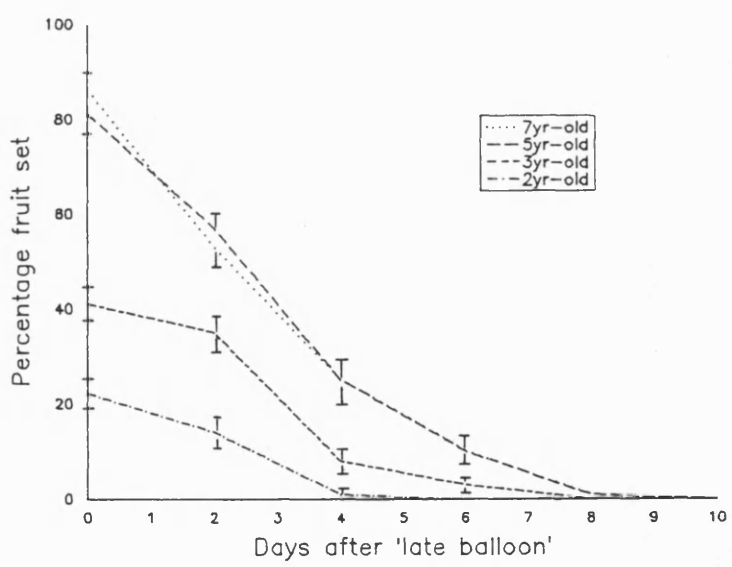
(a) horizontal branches
(b) vertical branches

pollinated at increasing lengths of time after 'late balloon'. Bars indicate S.E.M.

a) 1985



b) 1986



Figures 5.4.2.1a + b
Percentage fruit set of flowers borne on 2-year-old wood within various ages of tree pollinated at increasing lengths of time after 'late balloon' in;

(a) 1985
(b) 1986

Bars indicate S.E.M.

Percentage fruit set on the youngest trees was extremely low throughout the whole flowering period. Only 10% of flowers pollinated either at late balloon or 4 days later set fruit. Of flowers pollinated after this time, less than 2% set fruits.

In 1986, although the pattern of fruit set relative to tree age was not quite as consistent as in 1985, a trend of increasing fruit set with increasing tree age remained apparent throughout the entire pollination period (Figure 5.4.2.1b).

On the 4- and 6-year-old trees, the percentage fruit set obtained from pollination on each date were very similar. An initial set of 85% and 81% respectively declined rapidly such that only 19% and 24% of flowers on the same trees set fruit when pollinated 4 days after 'late balloon'. Two days later, fruit set was less than 15%. In comparison to these trees, percentage fruit sets on the 2- and 3-year-old trees were much poorer. On the latter, pollination at, or 2 days after 'late balloon' resulted in percentage fruit sets of 41% and 38% respectively. When pollination was delayed for a further 2 days only 8.1% of pollinated flowers set fruit.

On the 2-year-old trees, even lower levels of fruit set were obtained. Only 21% of flowers pollinated at 'late balloon' set fruit, and pollination four days later resulted in less than 1% of flowers setting fruit.

Where clusters had been defoliated at 'pink bud' various differences in size and setting ability were seen. The pedicel length and receptacle diameter of flowers from undefoliated clusters varied between the various tree ages but there was no relationship between the two variables, flowers from 3-year-old trees being larger than those from all other tree ages (Table 5.4.2.1). Similarly within the defoliated clusters, flowers from 3-year-old trees had larger receptacles, though similar pedicel lengths than did flowers from the other ages of tree. However, when the difference in size between the measured flowers from each pair of clusters was calculated, it was seen that within the 6- and 12-year-old trees defoliation had reduced pedicel length and receptacle diameter by about 3%, but that on the youngest trees defoliation reduced these parameters by more than 20% and 6% respectively. Flowers from the 4-year-old trees were intermediate to these and orientation did not affect this.

Fruit set was more affected by defoliation in the younger trees than the older ones. On the former, 10% of flowers on control clusters set fruit, none were set on the defoliated ones. Similarly on the 3-year-old trees, defoliation decreased fruit set by around 64%. In contrast, the set of defoliated clusters on the oldest trees slightly exceeded that of the controls, being 64.7% and 62.1% respectively. Clusters from 4- and 6-year-old trees were intermediate, defoliation reducing set by 30% and 10% respectively. Set on the vertical branches was slightly lower than on corresponding horizontal branches but defoliation did not apparently affect them differently.

Table 5.4.2.1 Pedicel length, receptacle diameter (and S.E.M.) and fruit set of flowers in control and defoliated clusters on various ages of tree. Clusters were on a 2-year-old horizontal wood on 2-, 3-, 4-, 6- and 12-year-old trees and 2-year-old vertical wood (V) on 4-year-old trees.

tree age	pedicel length (mm.)			receptacle diameter (mm.)			fruit set		
(years)	with leaves	defoliated	% decrease	with leaves	defoliated	% decrease	with leaves	defoliated	% decrease
2	9·36 (0·46)	7·45 (0·45)	20·6	3·02 (0·034)	2·81 (0·036)	6·9	10·2	0·0	-
3	11·75 (0·57)	9·0 (0·44)	24·0	3·44 (0·058)	3·22 (0·039)	6·5	51·3	18·7	63·5
4	10·06 (0·44)	8·94 (0·45)	11·4	3·08 (0·037)	2·94 (0·039)	4·6	59·1	42·4	28·3
4V	10·24 (0·57)	9·24 (0·47)	9·8	3·11 (0·040)	3·00 (0·047)	3·6	52·7	39·4	25·2
6	11·50 (0·068)	11·12 (0·54)	3·3	3·20 (0·050)	3·11 (0·050)	2·8	66·3	58·3	12·1
12	11·38 (0·33)	11·14 (0·42)	2·3	3·37 (0·034)	3·29 (0·034)	2·6	62·1	64·7	4·2

5.4.3 Experiment 3: Determination of the time period during which branch orientation exerts an influence over the fruit setting ability of flowers.

Within all branch orientation 'treatments' (ie. branches horizontal or vertical during either or both of the periods of floral initiation or flowering), it was seen that the values of percentage fruit set were highest when flowers were pollinated at the 'late balloon' stage, declining steadily as pollination was delayed, such that pollination 8 days after 'late balloon' resulted in virtually no fruits being set (Table 5.4.3.1).

Values of percentage fruit set resulting from each individual time of pollination were similar within flowers on either horizontal or tied-down branches.

Within both these situations approximately 74% of flowers pollinated at 'late balloon' set fruit. As pollination was increasingly delayed fruit set declined steadily such that only 8% of pollinations conducted 8 days after 'late balloon' resulted in fruit set (Table 5.4.3.1).

Although consistently lower than those described above, percentage fruit sets obtained within the remaining treatments followed a similar pattern. On the vertical or tied-up branches, similar percentages of flowers set fruit on each date of pollination. Within both situations, approximately 60% of flowers pollinated at 'late balloon' set fruit. As pollination was increasingly delayed, percentage fruit set rapidly declined such that when pollination occurred 4 days after 'late balloon' only 11% of flowers set fruit. On each date of pollination this was approximately half the percentage fruit set achieved by of flowers on horizontal, or tied-down branches.

Calculation of the percentage fruit set resulting from all pollinations conducted during the first 6 days (i.e. 4 pollinations) indicated that the highest values of percentage fruit set were obtained from flowers borne on either the horizontal or tied-down branches (43.6% and 42.2% respectively). Flowers on vertical or tied up branches set significantly fewer fruit (31.1% and 31.9% respectively). Differences in percentage fruit set over this time were significant between the vertical or tied-up branches compared to the horizontal or tied-down ones but not within these groupings.

5.4.4 Experiment 4: Determination of shoot tip influence on the quality of flowers borne on different orientations of wood, and assessment of the time period during which this influence operates.

Shoot growth arising from vertical 2-year-old branch units was significantly greater than that from similar horizontal branches units (Table 5.4.4.1). On average, in 1985 vertical and horizontal branches gave rise to approximately 128 and 86 cm of new shoot respectively.

On horizontal branches where shoot tips had been removed during August 1985, such treatment had not influenced total shoot production. On vertical branches however, removal of shoot tips was associated with a 15 cm reduction in total shoot growth compared to the untipped vertical branches.

Table 5.4.3.1. Percentage fruit set of flowers pollinated at different times after late balloon. Flowers were borne on 2-year-old wood on horizontal or vertical branches. The orientation of some branches had been reversed (horizontal → vertical and *vice versa*) 20 days prior to the first pollination. The average percentage fruit set obtained from pollination on days 0-6 inclusive is also given. Mean and (standard error) obtained using analysis of deviance with binomial model.

	branch orientation	(a) prior to April 10th (b) after April 10th		
date of pollination	(a)	horizontal		vertical
(days after late balloon)	(b)	horizontal	vertical	horizontal	vertical
0		74 · 97 (3 · 40)	58 · 85 (4 · 24)	73 · 41 (3 · 80)	59 · 96 (4 · 37)
2		63 · 14 (4 · 12)	44 · 98 (4 · 48)	61 · 21 (4 · 25)	46 · 13 (4 · 41)
4		20 · 35 (3 · 04)	10 · 87 (2 · 07)	19 · 06 (3 · 10)	11 · 33 (2 · 14)
6		8 · 88 (2 · 45)	4 · 45 (1 · 37)	8 · 24 (2 · 32)	4 · 65 (1 · 44)
8		0 · 08 (0 · 08)	0 · 05 (0 · 05)	0 · 06 (0 · 06)	0 · 0 -
average percentage fruit set obtained from pollination on days 0-6 combined		43 · 62 (2 · 45)	31 · 08 (2 · 46)	42 · 21 (2 · 61)	31 · 85 (2 · 51)

Table 5.4.4.1. Shoot growth arising from, and the number of fruit buds produced on branch units of 2-year-old wood growing in either a vertical or horizontal direction, with or without shoot tip removal.

branch orientation	horizontal		vertical	
tipping treatment	with tips	without tips	with tips	without tips
total shoot growth arising from branch unit (cm.) (S.E.M.)	82 · 8 (3 · 45)	89 · 1 (5 · 01)	136 · 1 (5 · 40)	121 · 4 (6 · 52)
number of shoots produced (S.E.M.)	2 · 51 (0 · 18)	2 · 42 (0 · 23)	3 · 70 (0 · 33)	3 · 76 (0 · 45)
mean shoot length (cm.) (S.E.M.)	37 · 15 (1 · 82)	37 · 60 (1 · 97)	41 · 75 (2 · 23)	38 · 55 (2 · 38)
fruit bud number (S.E.M.)	8 · 28 (0 · 58)	9 · 21 (0 · 71)	8 · 98 (0 · 67)	10 · 86 (0 · 86)

Table 5.4.4.2. Pearson correlation coefficients for components of shoot growth arising from 2-year-old branch units against the number of fruit bud produced on the same units in branches given various orientation/tipping treatments.

orientation/tipping	total shoot growth (cm.)	mean shoot length (cm.)	shoot number
horizontal branches	0 · 109	0 · 223	0 · 084
horizontal branches tipped in August 1985	−0 · 047	0 · 002	−0 · 066
vertical branches	−0 · 170	−0 · 120	0 · 219
vertical branches tipped in August 1985	−0 · 240	−0 · 039	−0 · 215

Shoot tip removal would usually be expected to halt shoot extension at least temporarily. Therefore it would appear that at the time of tipping, active shoot growth was occurring within the vertical, but not the horizontal branches. The number of shoots arising from each branch unit was affected by branch orientation but not shoot-tip removal (Table 5.4.4.1). Vertical branches produced an average of 3.7 new shoots during 1985 whereas horizontal branches produced only 2.5.

The average length of shoots on horizontal branch units (approximately 37 cm) was unaffected by shoot-tip removal, and was similar to that occurring on the vertical branches where shoot-tips had been removed. On the untipped vertical branches however, the mean length of shoot produced was 41.7 cm., significantly longer than those produced on horizontal untipped branches.

The numbers of fruit buds produced in 1986 on the 2-year-old branch units were higher, within both branch orientations, on tipped branches compared to untipped ones (Table 5.4.4.1), but these differences were not significant within the horizontal branches. Vertical tipped branches produced an average of 10.9 fruit buds each compared to 9.0 buds produced on untipped ones; horizontal tipped and untipped branches produced an average of 9.2 and 8.3 fruit buds respectively.

To investigate whether there was any relationship between any of the components of shoot growth and the number of fruit bud produced, the Pearson correlation coefficient between these characters was calculated within each treatment. However, no evidence for any relationship between these components was seen; neither total shoot growth, nor mean shoot length nor number of shoots correlated significantly with the numbers of fruit bud produced per branch (Table 5.4.4.2).

Assessment of female fertility and the length of EPP of flowers in these four situations plus those on horizontal and vertical branches where shoot tips had been removed in April 1986 showed that flowers borne on horizontal branches possessed a higher female fertility than did those borne on vertical branches (Table 5.4.4.3). More than 70% of flowers on horizontal branches set fruit when pollinated at 'late balloon' compared to between 43% and 52% of those on vertical branches. Although removal of shoot tips (either in August or April) appeared to increase percentage fruit set within both branch orientations, differences were not significant.

During this experiment, the length of the EPP within all treatments was very short, virtually no fruit being set by pollination 4 or more days after 'late balloon'. This was most apparent within the vertical branches where only 10% of flowers pollinated only 2 days after 'late balloon' set fruit. Pollination of flowers on horizontal branches resulted in slightly higher numbers of fruits set but was still unexpectedly low at between 24% and 30%. Thus the time during which pollination resulted in successful fruit set was very short.

Table 5.4.4.3. Percentage fruit set (and S.E.M.) of flowers borne on horizontal or vertical sections of 2-year-old wood which had had shoot tips removed in (a) August 1985, (b) April 1986 or (c) were untipped. Pollinations commenced at late balloon and continued at 2 day intervals.

		time of pollination (days after late balloon)			
branch orientation	shoot tips removed	0	2	4	6
horizontal	---	71 · 94 (4 · 25)	24 · 49 (3 · 56)	0 · 0 (0 · 0)	0 · 0 (0 · 0)
	August 1985	75 · 39 (3 · 33)	27 · 94 (3 · 10)	0 · 0 (0 · 0)	0 · 0 (0 · 0)
	April 1986	77 · 06 (3 · 19)	29 · 83 (3 · 41)	0 · 0 (0 · 0)	0 · 0 (0 · 0)
vertical	---	43 · 69 (4 · 68)	8 · 94 (1 · 74)	1 · 56 (1 · 56)	0 · 0 (0 · 0)
	August 1985	51 · 86 (5 · 23)	12 · 00 (2 · 27)	0 · 0 (0 · 0)	0 · 0 (0 · 0)
	April 1986	47 · 52 (5 · 13)	10 · 28 (3 · 41)	0 · 0 (0 · 0)	0 · 0 (0 · 0)

Averaged over the first two pollination times, it was seen that in general, percentage fruit set of flowers borne on horizontal branches was approximately double that obtained from those on vertical branches (Table 5.4.4.3). Shoot tip removal appeared to have no significant influence on the fruit setting abilities of flowers borne on branches of either orientation.

5.5 Discussion

Overall, the results described in this chapter indicate that flowers on very young trees, or vertical branches, consistently set fewer fruit per pollinated flower than do those on older trees or horizontal branches respectively. This supports the observation by many workers (Gardner *et al.* 1952, Forshey 1978, Dennis 1979) that although blossom is often formed on young trees, little fruit is produced. Similarly others have noted that trees with horizontal branches yield more fruit than do equivalent trees with more upright branches (Preston 1974).

Within these main results, three others stand out. Firstly, branch orientation appeared to exert a greater influence on fruit set during the period of spring flower development and bloom rather than during the period prior to this. Secondly, the age of wood within a branch (either horizontal or vertical) greatly influenced floral setting ability. And thirdly, removal of shoot tips during the time of flower initiation and differentiation or immediately prior to bloom had little effect on fruit set.

Within all cropping situations examined, flowers were most receptive to pollination at, or immediately after the 'late balloon' stage. As pollination was delayed beyond this, percentage fruit set declined. In the three years of study, the length of time after 'late balloon' during which pollination resulted in fruit set was around 8-10 days. However, between the individual situations and years there were differences in the pattern of changing floral receptivity to pollination and these will be discussed in the following sections.

5.5.1 Age of tree.

That young trees often fail to produce fruit is widely accepted (Williams 1972, Elfving and Forshey 1976, Greene and Lord 1978). It is often attributed to the vigorous shoot growth common in such trees (Forshey 1978, Williams 1983) and the accepted antagonism between it and fruit production (Lang 1961). Although there have been few detailed studies of the components of cropping within young trees, some reports state that although trees flower well, no fruit are produced (Gardner *et al.* 1952, Forshey 1978). The implication is that lack of fruit set or fruitlet retention is a greater problem than flower production, a premise supported by the results shown here, and in Chapter 2.

Differences in fruit set between the various tree ages were caused by a combination of inherently different levels of female fertility at anthesis and the rate at which this declined as time progressed. The female fertility of flowers on trees 4- or more years-old was such that

pollination at 'late balloon' resulted in between 58% and 73% of flowers setting fruit in both years, compared with only 11% (1985) or 21% (1986) within 2-year-old trees.

The effective pollination period (EPP) also varied between the different ages of tree. Measured as the period of time during which 10% or more of pollinated flowers set fruit, in 1985 EPP ranged between 8 and 9 days on the 3- to 12-year-old trees but was only about 5 days on the 2-year-old trees. In 1986 it was shorter, being about 6 days for 5- and 7-year-old trees, four for the 3-year-old trees, and less than three for the 2-year-old trees.

However, there are several points to bear in mind here. In 1985, flowers on 2-year-old trees developed later than did those on other ages of tree and consequently they reached the 'late balloon' stage approximately three days behind them. As such, all pollinations and assessments of set were correspondingly delayed by 3 days and were subject to different weather conditions. Female fertility and EPP are highly dependent on environmental conditions and temperature, wind, light and nutrition are all important.

Temperature is perhaps the most important (and variable) factor, capable of increasing or decreasing set greatly and many people have noted that 'warm, sunny weather during flowering' is beneficial for good fruit set (Thompson and Lui 1973, Lapins and Arndt 1974). Within the natural situation pollination is particularly susceptible to weather with bees rarely flying when temperatures drop below 10-14°C (see Dennis 1979). However, temperature still plays a large role even when flowers are hand pollinated. Fruit set is achieved when pollen germinates on a receptive stigma and the resultant pollen tube grows through the style to reach a healthy ovule. Increased temperatures can increase the rate at which pollen germinates (Deckers and Porreye 1984) and grows (Child 1967), a temperature rise from 7-12°C can reduce the time required for pollen tube growth through the style from 10 days to 5 days (Williams 1970b) and can therefore also increase fruit set. There are references to the fact that fruit set is poor in very cool years (Lapins and Arndt 1974) and this is often at least partly attributed to slow pollen tube growth (Thompson and Lui 1973). But increased temperatures can also reduce yield (Roberts 1947, Grauslund and Hansen 1975) High temperatures can cause floral desiccation and/or induce premature ovule degradation, both of which would then shorten the EPP (Dorsey 1929, Williams 1970). In 1985, the mean temperature over the flowering period of the older trees was 12.2°C; that for the younger trees was 13.4°C. Thus it is possible, if unlikely, that flowers from the young trees were being adversely affected by the slightly increased temperatures.

Wind has also been shown to inhibit fruit set. Hedrick (1908) observed wind damage - both mechanical and desiccating - within orchards, and several studies have since shown that the use of windbreaks within orchards can increase set (Srivastava 1938, Smith and Lewis 1972). One of the problems of using existing orchard material to conduct experiments investigating tree-age effects on fruiting is that they are almost invariably in different sites and may there-

fore be subjected to different environmental influences. Although the greatest care was taken to minimise such differences, because each age of tree was planted in separate orchards, some were inevitable. Most pertinent here is that the 2-year-old trees used in 1985 were part of a large planting, which due to its size and site, and the smallness of the trees, meant they were perhaps more exposed to the wind than were the other orchards. This might be expected to compound any adverse effects of high temperatures by enhancing their desiccating effects. Flower clusters used in these experiments were enclosed in glassine paper bags to prevent cross pollination. These bags reduce the risks of wind desiccation but they may increase mechanical damage. Initially they have enough rigidity to stand proud of the cluster, but when wet they lose this and may be blown against the flowers. Although some people have suggested that rain itself is detrimental to fruit set - causing pollen grains either to burst or else to be washed off the stigma (Hedrick 1908) - Beattie and Folley (1977) calculated that rainfall during flowering did not significantly affect Cox yields in Britain. However, within the experimental EPP situation, the combined effects of rain and wind may increase the chance of the glassine bags mechanically damaging the clusters. Such damage was seen in several clusters on the youngest trees and although any cluster thought to be damaged was not used, non-apparent damage may have contributed to reduced fruit set. However, another point to be borne in mind is the reason for the delayed flower development in the first place. Late flowering has previously been shown to be associated with poor 'quality' flowers - that is - flowers with low female fertility and/or short EPP (Buszard 1983). One reason suggested for the delay is that such flowers had been initiated later in the previous season than had other, earlier, flowers and as such had not developed as far or as well by the time dormancy set in (Abbott 1984). These flowers would then either have more development to do the following spring, or may perhaps just have less stored resources to draw upon then. This would certainly be consistent with the suggestions that a) shoots on young trees grow more rapidly and until later than do those on older trees (Williams 1983 and Chapters 3 and 7), and b) that floral initiation is delayed in vigorously growing shoots (Luckwill 1970). However, although the growing tip undoubtedly exerts an influence on floral initiation within axillary buds, whether or not this extends to initiation within spurs is less certain. Of no doubt though, is that the presence of fruit on a spur and the gibberellin which diffuses from its seeds, is inhibitory to flower initiation (Hoad 1978). In the circumstances here however, it was the youngest trees which flowered late and had poor fruit set, yet these trees had borne virtually no fruit the previous year. It would appear therefore that in this case, and perhaps in all spurs on 2-year-old wood, the growing shoot tip does exert considerable influence on flowering and subsequent fruit set.

However, in 1986 the situation was different. Floral development on all ages of tree proceeded at the same rate, approximately 50% of flowers on all trees reaching the 'late bal-

loon' stage on the same date. Subsequent pollinations were therefore carried out over common time periods and weather conditions. As seen in 1985, fruit set on the 2- and 3-year-old trees was again very poor and they had both lower female fertility on every date of pollination and a shorter EPP than did flowers from the older trees. This suggests that the 1985 results did present a true, if possibly exaggerated picture of the fruit setting abilities of flowers on trees of different ages. In both years the main differences in female fertility and EPP were that on the 2- and 3-year-old trees these were both much lower than on all the other tree ages. Within trees aged 4-years or more, few major differences were seen in either parameter.

It can be concluded from this that for some reason(s), flowers from young trees (2-, 3- and sometimes 4-year-olds) have a lower female fertility at anthesis than do those from older trees. This presumably is due to one or a combination of low stigmatic receptivity, poor pollen tube growth, ovule abnormalities or early embryo abortion. Female fertility also declines more rapidly in these flowers than in those from older trees. Further investigations to determine which of these are most important are reported in Chapter 6.

Regarding the setting ability of flowers on clusters defoliated at 'pink bud' it was seen that this treatment reduced flower size and set more on the young trees than it did on the older ones. Presumably, until new leaves are grown and these start exporting assimilates, the flowers on defoliated clusters are dependent upon resources drawn from the tree. The greater percentage decrease in size of flowers on the younger trees may reflect a lower ability to obtain resources in the absence of leaves. This could be because these flowers only have a low 'sink' strength and cannot attract nutrients towards them as well as can flowers on older trees, alternatively it may reflect a situation where stored reserves within the tree are low, and the flowers would normally be more dependent on their cluster leaves for nutritional supplies. If this is the case then the consistently smaller leaf area seen in the clusters from young trees (Chapter 4) may suggest a reason for their lower levels of fruit set.

5.5.2 Branch orientation

There are reports in the literature suggesting that trees with horizontal branches produce more fruit than do those with a more vertical habit (Preston 1974) an effect often attributed to the greater flower production observed on the former (Tromp 1968, 1972, 1987). Training branches to a horizontal position tends to decrease their vigour and increase their flower production (Wareing and Nasr 1958, Tromp 1970, 1972). Although it has been recommended for improving the set of young trees (Dennis 1979, Greene 1981) it is not always successful (Greene and Lord 1978). Results here show that in each of the three years of study, flowers on horizontal branches consistently set better than did those on vertical branches. Several possible reasons for this could be suggested.

Many people have noted an inverse relationship between rate/amount of shoot growth and fruit set (Abbott 1960, Forshey 1978, Hansen 1980) and conditions which induce vigour (e.g. high fertilisation, heavy pruning) can cause a decrease in flower production and fruit set (Batjer and Westwood 1963, Elfving and Forshey 1976).

Similarly, treatments which reduce vigour (shoot tipping, chemical pinching agents, plant growth regulators) can all successfully increase flowering and fruiting. It has been shown that flowers and/or fruitlets are in competition with shoot tips for nutrients and that techniques which reduce this competition can increase fruit set (Abbott 1960, Quinlan and Preston 1971) Horizontal orientation of shoots is associated with a decrease in apical dominance (Wareing and Nasr 1961), and a resultant decrease in the vigour of shoot growth (Kato and Ito 1962, Elfving and Forshey 1976). If this vigour is reduced during flowering then a horizontal orientation may influence fruit set by lowering apical dominance and the competitive effect of the shoot tip.

It has also been suggested that conditions during the time of flower initiation and differentiation the previous year can affect fruit set (Williams 1965, Williams *et al.* 1980). Timing of flower initiation (Abbott 1970), or the level of competition for available resources (Williams *et al.* 1980) might affect the state of the bud as it enters dormancy and thereby affect its quality the following year.

Horizontal orientation of branches has been shown to cause earlier shoot growth cessation in comparison to vertical ones (Kato and Ito 1962, Tromp 1968 and Chapter 7) and although the exact relationship of the timing of this and flower initiation is disputed, there are many reports suggesting that the two occur around the same time (see Buban and Faust 1982). If this is the case, a horizontal orientation which induces early cessation of growth may advance the time of flower initiation within its buds. This would allow them to develop further before dormancy (Abbott 1984) and by doing so, perhaps improve bud quality and increase fruit set the following year. These possibilities are investigated and discussed in Chapter 7. However at this point it is pertinent to note that results from Experiment 3 suggest that branch orientation during the period of flowering itself has a greater effect on fruit set than it has during the autumn prior to it. Compared to vertical branches, tying to a horizontal orientation in April increased the level of female fertility and length of EPP within flowers to levels equivalent to those found in flowers on branches kept horizontal throughout. Similarly, tying up initially horizontal branches in April reduced the level of female fertility and length of EPP to that found on branches which had been vertical throughout.

This would suggest that if shoot growth competition is a major factor determining fruit setting ability, then it exerts its influence during, and immediately prior to, flowering rather than during the period of flower initiation and differentiation the autumn before. Forshey (1978) reported growth to start earlier, and proceed at a greater rate on vertical branches compared

to horizontal ones; this situation would place flowers on the vertical branches under a greater competitive pressure for metabolites than were those on horizontal branches and might therefore affect their setting ability.

When the question of shoot tip influence on fruit set was approached in another way, by trying to remove it by tip excision, during either flower initiation and differentiation or flower development and bloom, no shoot tip effects were seen. Once again, flowers on horizontal branches had higher female fertility and longer EPP than did those from vertical branches but no effects of shoot tip removal were superimposed on this. This might therefore suggest that the enhancement of fruit set seen on the horizontal branches in this and the previous experiment are due to direct orientational effects rather than indirect ones via changes in shoot tip competition.

5.5.3 Age of wood

Flowers on 1-year-old wood set less well than did those on older wood. Although some varieties are exceptions to this, lack of set on 1-year-old wood is a common occurrence (May 1972). Several reasons have been put forward to account for this, one of which is very similar to the possible explanation of poor fruit set on young trees. It suggests that because flower initiation occurs later on this wood than on older wood (Zeller 1960), the buds are less developed with fewer stored resources when they enter winter dormancy (Abbott 1984). Growing shoot tips exert apical dominance over lateral buds on the shoot (Wareing and Nasr 1961), and because 1-year-old wood is closer to the growing tip than is the older wood, it might be expected to be under greater control. Whilst lateral buds are suppressed by apical dominance, they will develop only slowly and may not even start floral initiation. Buds further away from the tip and the source of inhibition (i.e. on older wood) can have greater more meristematic active and may therefore reach sufficient complexity to initiate flowers relatively early. Floral initiation occurring early in the season will allow the bud to reach an advanced state of development and/or store nutrients prior to winter dormancy and have a better setting ability the following year (Abbott 1970).

That the length of time between flower initiation and dormancy is important for flower quality was clearly shown by Abbott (1970). He regulated the amount of time trees had between initiating flower buds and dormancy and thus produced buds of different 'ages' entering the dormant phase. When dormancy was broken and the flower buds developed, the ones which had only had a short period of time between initiation and dormancy were 'weaker' than those which had had longer. This was expressed as the 'older' buds having a higher female fertility than did the 'younger' ones.

However, perhaps more surprising is the result showing differences in fruit set on 2- and 3-year-old wood. The general presumption was that flowers on older wood would set equally

well. But, although differences in set were small, flowers on 2- year-old wood consistently and significantly set more fruit than did those on 3-year-old wood. This obviously does not fit in with the above theory about the timing of flower initiation and consequent bud age re distance from the growing shoot tip; indeed, using that argument, buds on 3-year-old wood might be expected to initiate before those on 2-year-old wood. No other work appears to have been done directly on this subject but it is interesting to note that in a recent paper (Velickovic and Jovanovic 1987) fruit quality was found to differ depending on the age of wood on which it it was borne. Once again differences between 2- and 3-year-old wood were identified, the best fruit being a product of the former. Both results could be due to the greater possibility of the 3-year-old wood having borne fruit the previous year, the presence of which might have affected fruit production the following year in two ways. Firstly seeds being rich sources of gibberellins (Dennis and Nitsch 1966) and proved to be strong inhibitors of flower initiation (Chan and Cain 1967, Hoad 1978), may therefore have caused flower initiation to be delayed and/or have had a depressive effect on the stage of development reached by the flower bud prior to winter dormancy. Secondly, the presence of fruits would compete with the branch itself for any nutrients available for storage, and therefore the following year, less reserves may have been available to support cluster development and fruit set (Williams *et al.* 1980).

Although differences in the fruit set obtained from flowers on 2- and 3-year-old wood were small, the differences were real. It may be that the use of pruning systems which maximise the retention of 2-year-old wood at the expense of other ages could provide a means of enhancing fruit set on these trees.

Chapter 6. Anatomy and physiology of flowers during the flowering period : influence of tree age, wood age, and branch orientation.

6.1 Introduction

Apple flowers are epigynous, all flower parts arising above the ovules, and the fruit is the enlarged receptacle with enclosed pistils. In Cox most flowers have 5 loculi each with two carpels containing a single ovule, giving a total of 10 embryo sacs and 10 potential seeds. The normal number of ovules found in apple is characteristic of the variety and the environmental conditions under which it is grown. Although the absolute number of seeds in a fruit is not crucial, and the retention and growth of fruits containing one seed can be equivalent to those with 10 seeds (Abbott 1984), where there is competition with other fruitlets or with vegetative growth, fruits lacking seeds or having very low seed numbers, tend to abscise prematurely (Luckwill 1948), or if retained until maturity, are small in size (Crane 1964). It follows therefore that the fertilisation of all embryo sacs, and the subsequent development of the ovule into seeds is an important factor in ensuring successful fruit set.

Fertilisation requires pollination and passage of male gametes to the egg sac. For this to happen, stigmas must provide a receptive surface for pollen adhesion and germination. Strong stylar tissue may be necessary to provide nourishment for growing pollen tubes, and egg sacs must remain viable long enough for pollen tubes to reach them. Stigmas and egg sac both have limited life and the duration of these components is likely to be a function of the physiological and environmental conditions of the fruit tree.

The formation of a fruitlet is thus a complex process offering several opportunities for failure. Although only one part of the process need break down to prevent fruit-set, in practice it is likely that a flower which fails to set will be inadequate in several aspects.

Breakdown has been observed in several components of the fruit setting process. It has been reported that the condition of the stigma and style changes with time after anthesis; this being seen as a general browning of the style (Dorsey 1929), collapsing stigmatic papillae (Braun and Stosser 1985, Miller 1988), or degradation of accumulated starch within the style (Stosser and Neubeller 1980). Differences in stylar condition and possible receptiveness to pollen germination and growth have been seen in trees with various crop loads (Buszard 1983), trees subjected to differing spring temperatures (Miller 1988) and trees given different nitrogen treatments (Williams 1965). In the latter case the stigmas of 'strong' and normal flowers were compared and it was seen that while the stigmas of normal flowers could support pollen penetration up to eight days after anthesis, those of 'strong' flowers could do so for a further four days.

However, Braun and Stosser (1985) found that although stylar tissue apparently degenerated soon after anthesis, pollen continued to germinate on the stigma, and pollen tubes grew

through the style for several days beyond this. They concluded that in this situation, ovular condition was the factor limiting set. Similar conclusions have been drawn by several workers (Dorsey 1929, Williams 1965, Miller 1988). As outlined in Chapter 5, healthy embryo sacs are crucial for fruit set. Although Hartman and Howlett (1954) found no evidence to suggest that the quality of the embryo sac varied between flowers of differing 'strengths' Dorsey (1929) found that flowers of varieties which set badly tend to have a greater proportion of malformed embryo sacs than did those from varieties which usually set more readily. Many workers have concluded that differences in embryo sac longevity can be the primary reason for the variation in EPP which distinguishes 'strong' from 'weak' flowers (Williams 1965, Braun and Stosser 1985, Miller 1988).

However, even if fertilisation does occur, fruit set is not guaranteed, and some ovules grow only slightly before aborting (Luckwill 1953) - presumably due either to genetic problems or lack of post-fertilisation support.

It has been shown (Chapter 5) that within apple flowers, both female fertility and the length of EPP can vary significantly depending upon the age of tree, age of wood or orientation of the branch upon which a flower is borne. In order to investigate more closely the underlying reasons for this, the developing anatomy and condition of flowers borne within these differing situations was studied in detail. To this end, flowers were harvested during the period from 'late balloon' to approximately 10 days after anthesis and assessments were made of;

a) Receptacle diameter and pedicel length,
b) Stigmatic condition,
c) Pollen tube growth through the style,
d) Ovule condition at anthesis, and the rate of its degeneration in
i) unpollinated flowers and
ii) flowers pollinated at increasing lengths of time after anthesis,
e) Embryo development 14 days after pollinations which occurred at increasing lengths of time after anthesis.

6.2 Materials and methods

6.2.1 1985

During the 1985 EPP experiment described in Section 5.2.4 flower clusters from 2-year-old horizontal wood on 2-, 3-, 4-, 6- and 12-year-old trees, and on horizontal and vertical branches of 4-year-old trees were thinned at the 'late balloon' stage to five similar laterals, and then enclosed in glassine bags to prevent cross pollination. On the day of bagging, and every two days subsequent to this for 10 days, 20 clusters of each treatment were uncovered, one lateral harvested, and the remaining four hand pollinated prior to rebagging. Harvested flowers

were placed in polythene bags within an insulated box and transported back to the laboratory for immediate examination.

Individual flowers were held under a Nachett NF50 binocular microscope and the topography of the stigmatic surfaces examined at magnifications between 10x and 40x. From this it was possible to observe the proportion of stigmatic papillae which were fully turgid, those which had collapsed, any discolouration present and the amount of any liquid present on the surface. Individual stigmas were scored using the categories below and means calculated.

(5) 100% papillae fully turgid
(4) 75 - 99% papillae fully turgid
(3) 50 - 74% papillae fully turgid
(2) 25 - 49% papillae fully turgid
(1) less than 25% papillae fully turgid
(0) no papillae fully turgid

A few representative stigmas were examined using a Cambridge Stereoscan 100 scanning electron microscope. Styles were mounted onto stubs using colloidal graphite, then quick-frozen in Nitrogen slush using a Hexland Cryo Transfer system. Surface frosting gained during transfer from slushing chamber to cryo stage was sublimed off at -70°C. Samples were sputter coated with gold at 6V for 3 minutes then transferred to the microscope stage which was maintained at -140°C. An accelerating voltage of 15V was used to examine the styles.

Immediately after completing the observation of the stigmatic papillae, pedicel length was measured to an accuracy of 1 millimetre, and receptacle diameter (at the widest point) measured to the nearest 0.05 mm using Camlab calipers.

Receptacles of the excised flowers were then prepared for histological examination. Receptacles with pedicels removed were scraped to remove epidermal hairs and samples were fixed in 4% formalin in 0.1M phosphate buffer at pH 6.8 *in vacuo* for 24 hours. After storage for 24 hours or longer, samples were rinsed in water, dehydrated in an ethanol/propan-1-ol series, and finally embedded in paraffin wax. Sections 10 µm thick, cut on a rotary microtome, were mounted on microscope slides using Haupts adhesive and 4% formaldehyde. Wax was removed in xylene and sections brought to the required state of hydration prior to staining with Haedenhains' haematoxalin. Samples were examined using a Leitz dialux microscope at magnifications between 16x and 40x and the condition of the embryo sac visually assessed. Because development and degeneration of the embryo sac are continuous, rather than discrete processes, categorising their condition at any given time is bound to be subjective, and exact classification difficult. Therefore, in both years, ovules were classified as being either (i) immature (either having less than 8 nuclei or the embryo sac not having expanded), (ii) mature and healthy (showing an expanded nucellus and integuments, and well organised embryo sac), or (iii) overmature (contents of the embryo sac cytoplasm withdrawn, densely stained

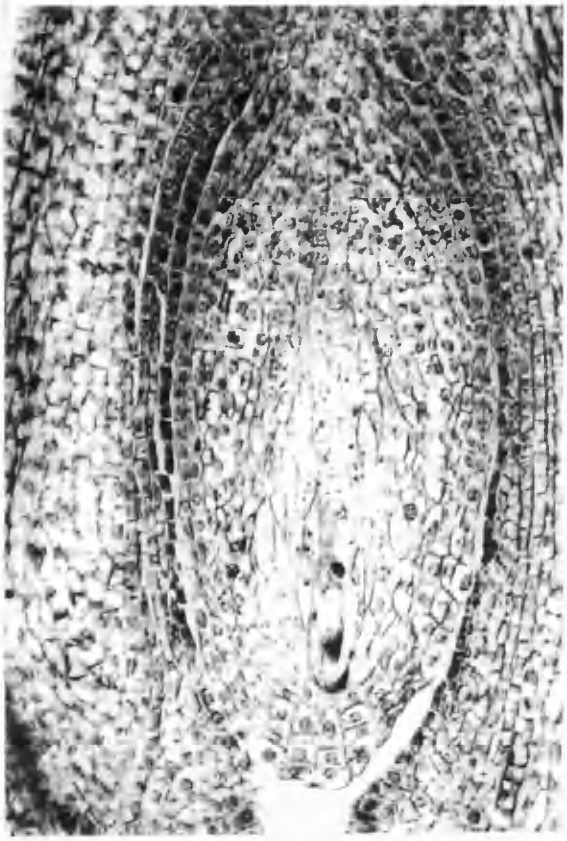
Plate 6.2.1.1 Longitudinal section through ovules of Cox flowers showing egg-sacs in various stages of maturity.

(a) and (b) immature with 2 and 4 nuclei respectively

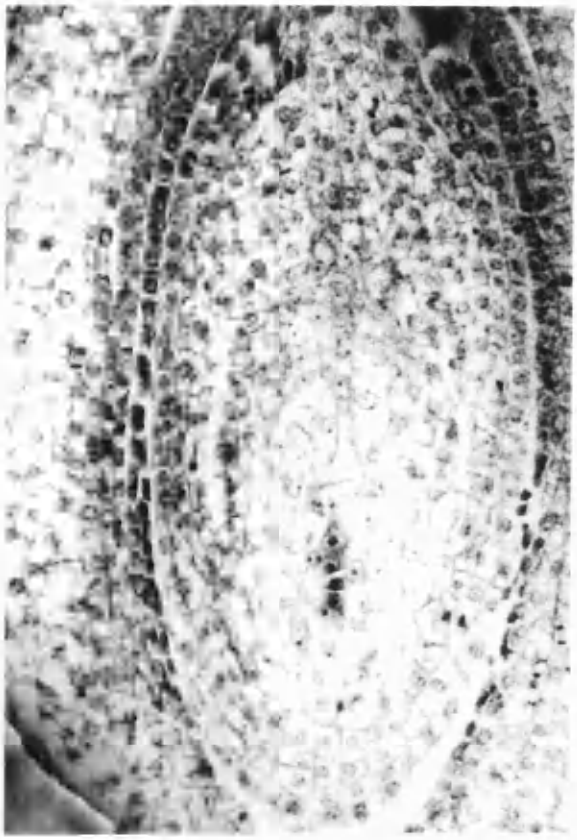
(c) mature

(d) degenerating.

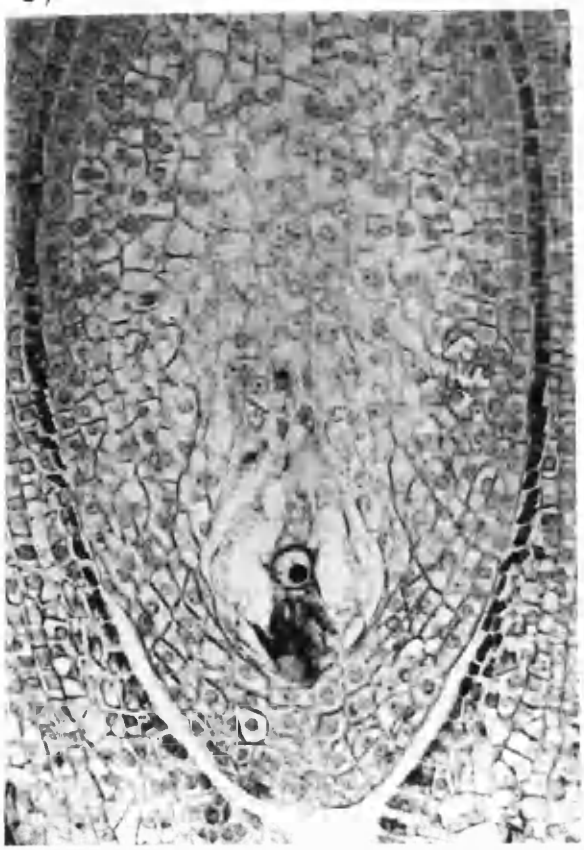
a)



b)



c)



d)

and disorganised) as shown in Plate 6.2.1.1. Occasionally, in overmature ovules, the nucellus and/or integuments were shrunken, degenerate and pulled away from the receptacle. Contingency tables were used to analyse the data.

In 1985, pollen tube growth within flowers *in situ* on 2-year-old wood within 2-, 3-, 4-, 6- and 12-year-old trees was examined. On the 4-year-old trees, flowers borne on 2-year-old vertical wood were also examined. Two clusters on each of 10 trees from each situation were thinned at the 'late balloon' stage to four laterals, emasculated and enclosed in glassine bags. This occurred on May 12th for all but the youngest trees, where floral development, and subsequently, emasculation and bagging was delayed for approximately 3 days. 24 hours after bagging, the flowers were hand pollinated and the glassine bags replaced. Half the clusters (one per tree) from each situation were harvested 48 hours after pollination; the remainder 48 hours later. Styles were removed from the flowers, fixed in 5% sodium sulphite and autoclaved for 25 minutes before storage (Currier 1957).

For examination, styles were placed on a microscope slide and gently separated. These were flooded with 0.1% aniline blue stain, warmed slightly over a flame to aid stain absorption, then squashed under a coverslip. Preparations were examined using a Leitz dialux 20 microscope with NPL objectives and a dark blue filter set up for a dark ground image. Under such conditions the callose, which lines and plugs pollen grains and tubes, fluoresces green/yellow against a grey background and permits easy identification of pollen tubes.

The number of pollen tubes at four positions down the length of the style were recorded. 12 flowers (c 60 styles) were examined in each case. Due to the large quantities involved, exact counts of the number of pollen tubes initially penetrating the style were extremely difficult to do accurately; hence counts were made at several sites further down the style as shown in Figure 6.2.1.1.

6.2.2 1986

In 1986, pollen tube growth and ovule degradation were again examined in various cropping situations but it was considered necessary to first make some modifications to the 1985 experimental technique. In 1985, due to the lack of synchrony of flowering, flowers used to assess pollen tube growth rate were pollinated three days later on the youngest trees compared to the older trees. Because the rate of pollen tube growth through the style is affected by temperature (Williams 1970b), any difference in daily temperature between two measurement periods make results difficult to interpret. Also, although ovule degradation had been observed in unpollinated flowers during the blossom period of 1985, this might not be representative of the situation arising if pollination had occurred.

Consequently, in 1986 an experiment to investigate pollen tube growth, embryo sac degeneration and embryo development was designed to overcome these problems.

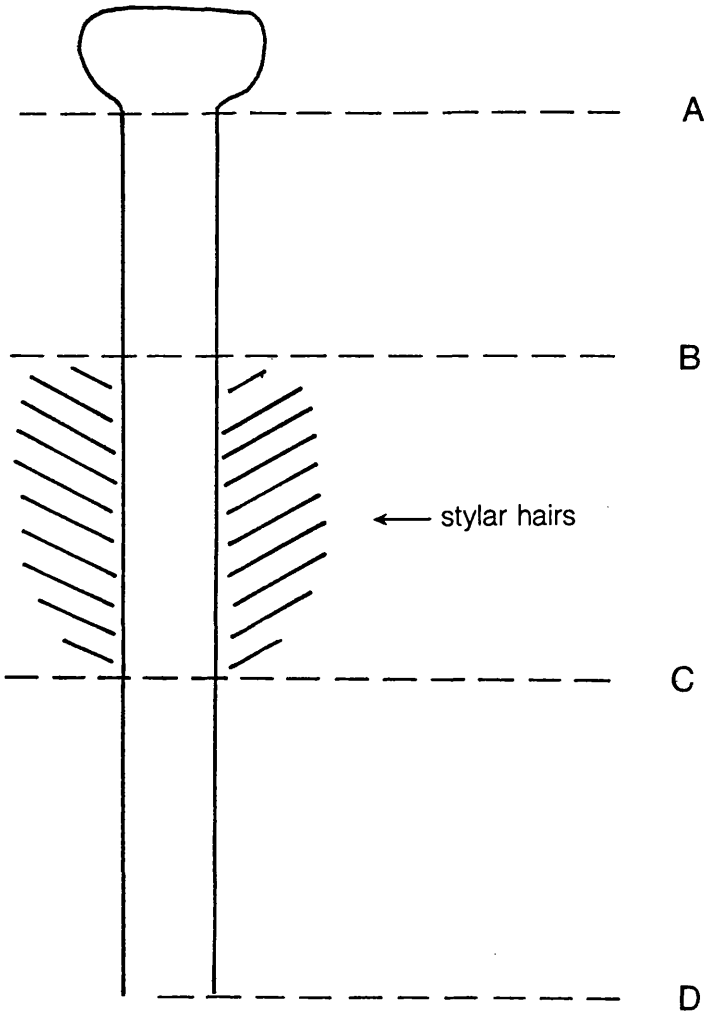


Figure 6.2.1.1 Schematic diagram of Cox stigma and style showing the four positions A, B, C and D at which pollen tube counts were made.

Pollen tube growth was studied *in vitro*, whereby regardless of the day of opening, flowers could be maintained in identical conditions at and after pollination. Ovule degradation and/or embryo development were examined in flowers which had been pollinated at various, predetermined times after full bloom.

These studies were conducted using the same trees as the EPP investigations described in Section 5.2.4. Various situations where differences in fruit setting ability had previously been observed, or might be expected to occur were examined. These were (a) different ages of tree, (b) different ages of wood within a tree, and (c) different orientations of branch within a tree as described below. Two-year-old wood was used throughout except in (vi).

(i) 2-year-old tree, horizontal wood,
(ii) 3-year-old tree, horizontal wood,
(iii) 5-year-old tree, horizontal wood,
(iv) 7-year-old tree, horizontal wood,
(v) 5-year-old tree, vertical wood,
(vi) 5-year-old tree, flowers harvested from midway along 1985 extension growth (i.e. axillary flowers),
(vii) 5-year-old tree, branches horizontal prior to 20th April 1986, vertical thereafter,
(iix) 5-year-old tree, branches vertical prior to 20th April 1986, horizontal thereafter.

For each treatment, six clusters on each of 12 trees were selected when laterals were at the 'late balloon' stage, thinned to five laterals, emasculated and enclosed in glassine bags. On the day of bagging and every two days for 10 days subsequent to this, one cluster per tree (12 per treatment) was selected at random. One flower was excised, the remaining four hand pollinated and re-enclosed within the bag. Excised flowers were transported in polythene bags within an insulated container back to the laboratory.

In the laboratory, the petals of each flower were removed (to minimise water loss) then the flowers were gently inserted into prepared holes in damp 'Oasis'. As rapidly as possible, and using the same techniques as in 1985, individual stigmatic surfaces were examined and categorised, and pedicel lengths and receptacle diameters measured. Flowers were then hand pollinated using stored pollen, and placed in an incubator at 15°C. After 48 hours incubation, the pedicel, sepals and anther filaments were removed from the flowers, the receptacles scraped clean of epidermal hairs and the resultant style and receptacle fixed in 5% sodium sulphite and autoclaved immediately for 25 minutes.

The staining technique used in 1985 had proved reasonably satisfactory but occasionally fluorescence 'faded out' towards the end of the pollen tube, making it difficult to identify the position of the growing point. Consequently, in 1986, a slightly different technique, adapted from Martin (1958) was used. Styles and attached receptacles were removed from the sodium sulphite solution, rinsed in distilled water for 30 minutes then placed in a solution of 0.1%

water soluble aniline blue dye in 0.1N K_3PO_4 for one hour. They were then placed on a microscope slide and moistened with 1-2 drops of staining solution. Excess receptacle tissue was gently removed, and whilst ensuring that ovaries were left in position at their base, individual styles were gently separated. The preparations were crushed gently under a coverslip and examined as in 1985.

The number of pollen tubes visible at positions down the length of the style were counted; 12 flowers (c 60 styles) were examined from each situation.

Of the remaining four flowers on each cluster used for the pollen tube growth experiment, 2 were harvested 96 hours after pollination; the remainder 10 days later. Receptacles were scraped free of epidermal hair, trimmed of excess cortex surrounding the ovaries and then fixed in Formalin Acetic Alcohol (FAA). Sections were cut and examined as in 1985.

6.3 Results

6.3.1 Flower size

In both 1985 and 1986, pedicel length tended to increase throughout the flowering period (Table 6.3.1.1). Within all ages of tree, pedicel length was significantly longer in 1986 compared to 1985. Although differences in pedicel length existed between trees of different ages, these were not consistent with increasing or decreasing tree age. In 1985, flowers from 2-year-old trees had the shortest pedicels (c 7mm), those from 6-year-old trees the longest (c 11mm), and those from 3-, 4- and 12-year-old trees were intermediate. In 1986, flowers from 5-year-old trees had significantly longer pedicels (c 16mm) than did those from any other age of tree (c 13-14mm).

Within the 5-year-old trees, flowers borne on 1-year-wood had significantly shorter pedicels than those borne on 2-year-old wood (Table 6.3.1.2a), but no branch orientation effects were observed other than that flowers from branches vertical prior to flowering and horizontal thereafter tended to have shorter pedicels than did those in other situations.

Receptacle diameter of flowers from all ages of tree and orientations of wood generally increased during the few days immediately following 'late balloon' and then remained relatively constant (Table 6.3.1.3 and 6.3.1.2b). In both years, flowers from the youngest trees had smaller receptacles (2.76 and 2.86 mm in 1985 and 1986 respectively) than did those from other ages of tree. In 1985 no other differences existed between flowers from the other tree ages but in 1986, flowers from 3-year-old trees also had significantly smaller receptacles than did those from 5- and 7-year-olds.

Within the 5-year-old trees, flowers from the different branch orientation treatments all had very similar receptacle diameters, but those from 1-year-old wood were significantly smaller than those from the 2-year-old wood (Table 6.3.1.2b).

Tables 6.3.1.1a + b Pedicel length (mm) of flowers borne on various ages of tree at late balloon and at intervals thereafter in (a) 1985 and (b) 1986. Mean values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

(a) 1985

days after late balloon	tree age (years) 2	3	4	6	12	date mean
0	5·8	8·9	9·0	8·6	7·6	7·9a
2	6·1	7·5	8·5	11·0	8·8	8·5a
4	7·3	7·1	8·5	11·2	7·7	8·4a
6	6·1	7·4	8·9	11·2	8·9	8·6a
8	7·8	10·4	10·2	13·1	12·3	10·8b
10	8·8	11·5	9·4	10·9	11·1	10·3b
age mean	7·0a	8·8b	9·1b	11·0c	9·4b	

S.E.D.: Age × Days = 0·99; Age = 0·41; Days = 0·42.

(b) 1986

days after late balloon	tree age (years) 2	3	5	7	date mean
0	12·2	11·3	14·9	13·0	12·8a
2	12·0	14·7	15·4	12·6	13·7bc
4	13·2	14·6	16·3	12·9	14·2cd
6	14·2	14·8	16·2	14·8	15·0d
8	13·4	14·3	17·2	14·5	14·8d
age mean	13·0a	13·9b	16.0c	13.6ab	

S.E.D.: Age × Days = 0·80; Age = 0·36; Days = 0·40.

Tables 6.3.1.2a + b (a) Pedicel length and receptacle diameter of flowers borne within branch orientation treatments, and on different ages of wood within a branch at late balloon and at intervals thereafter. Mean values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

Treatments were:-

H = 2-year-old horizontal wood
V = 2-year-old vertical wood
Ha = 2-year-old wood vertical prior to flowering, horizontal thereafter
Va = 2-year-old wood horizontal prior to flowering, vertical thereafter
Hi = 1-year-old horizontal wood

(a) pedicel length (mm.)

days after late balloon	branch orientation/wood age H	V	Ha	Va	Hi	date mean
0	14 · 1	11 · 0	13 · 4	15 · 0	13 · 2	13 · 3a
2	15 · 4	17 · 3	12 · 4	16 · 2	14 · 3	15 · 1b
4	16 · 3	16 · 8	16 · 3	17 · 7	12 · 6	15 · 9bc
6	16 · 2	18 · 5	16 · 8	17 · 6	12 · 2	16 · 3c
8	17 · 2	18 · 3	15 · 7	16 · 7	14 · 0	16 · 4c
age mean	15 · 8c	16 · 4c	14 · 9b	16 · 6c	13 · 2a	

S.E.D.: Treatments × Days = 0 · 104; Treatments = 0 · 40; Days = 0 · 41.

(b) receptacle diameter (mm.)

days after late balloon	branch orientation/wood age H	V	Ha	Va	Hi	date mean
0	3 · 11	2 · 95	3 · 12	3 · 07	2 · 84	3 · 06a
2	3 · 14	3 · 12	3 · 15	3 · 20	2 · 85	3 · 15b
4	3 · 17	3 · 20	3 · 18	3 · 29	2 · 85	3 · 13b
6	3 · 21	3 · 39	3 · 24	3 · 24	2 · 69	3 · 16b
8	3 · 23	3 · 27	2 · 93	3 · 16	2 · 71	3 · 07a
age mean	3 · 17bc	3 · 18c	3 · 12b	3 · 19c	2 · 78a	

S.E.D.: Treatments × Days = 0 · 063; Treatments = 0 · 029; Days = 0 · 032.

Tables 6.3.1.3a + b Receptacle diameter (mm) of flowers borne on various ages of tree at late balloon and at intervals thereafter in (a) 1985 and (b) 1986. Mean values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

(a) 1985

days after late balloon	tree age (years) 2	3	4	6	12	date mean
0	2·65	2·93	3·18	2·95	3·05	2·93a
2	2·79	2·98	3·14	3·07	3·02	2·98ab
4	2·64	3·13	3·09	3·01	2·95	2·94a
6	2·72	3·10	3·00	3·23	3·08	3·01ab
8	2·99	3·19	3·03	3·33	3·06	3·12b
10	2·78	3·15	2·99	3·22	3·13	3·05b
age mean	2·76a	3·13b	3·07b	3·13b	3·05b	

S.E.D.: Age × Days = 0·102; Age = 0·042; Days = 0·042.

(b) 1986

days after late balloon	tree age (years) 2	3	5	7	date mean
0	2·84	2·82	3·11	3·01	2·94a
2	2·79	2·95	3·14	3·00	2·97a
4	2·91	2·97	3·17	3·15	3·05b
6	2·85	2·99	3·21	3·24	3·07b
8	2·90	2·90	3·23	3·18	3·05b
age mean	2·86a	2·92a	3·17b	3·11b	

S.E.D.: Age × Days = 0·067; Age = 0·030; Days = 0·034.

6.3.2 Stigmatic surfaces.

In Cox, the stigmatic surface is a glandular epidermis of cells, papillate in shape, which are covered by a lipid protein pellicle and secrete a sugary liquid. At anthesis these cells are usually round and turgid, but after pollination has taken place, the papillae collapse, and pollen tubes penetrate the pellicle prior to growing down the style. In both 1985 and 1986, microscopic examination of apple flower stigmas indicated that as time after anthesis increased, papillae within unpollinated flowers began to collapse and shrink, and/or become enveloped by an accumulation of secreted liquid (Plate 6.3.2.1).

Scoring of these stigmas according to the proportion of papillae still turgid and not submerged by liquid, indicated that in both years the highest proportion of papillae were expanded and turgid on stigmas from flowers which had recently fully opened (i.e. the first harvest date) (Fig 6.3.2.1). As time after this increased, increasingly more papillae collapsed or became submerged, such that by 8, (1986) or 10, (1985) days after 'late balloon', on average less than 20% of papillae remained 'healthy'.

Although the pattern of stigmatic degeneration was similar in both years, (increasing steadily with time after anthesis), the nature of the degradation varied between years. In 1985, independent of tree age flowers harvested up to 4 days after 'late ballon' had styles which were green, and to the eye apparently healthy. As time after anthesis increased, slight browning of the stigma became apparent but styles remained apparently turgid and microscopic examination was necessary in order to determine and assess any stigmatic degeneration. In 1986 however, although when flowers were collected close to anthesis the styles appeared healthy, general necrosis occasionally became visible four or more days later. Initially this appeared, as in 1985, as a general brown tinge to the papillae of a few stigmas. As time progressed, the incidence and degree of this browning rapidly increased such that by 8 days after 'late balloon' many flowers had dark brown and apparently desiccated styles, with damage extending almost to the style base (Plate 6.3.2.2). In both years of study, stigmas were scored according to the proportion of healthy papillae present, independent of the nature of any degeneration.

In 1986, the rate of stigmatic degeneration over the flowering period appeared unaffected by tree age (Fig 6.3.2.1b). At 'late balloon', within flowers from all ages of tree, c 90% of papillae appeared healthy. This proportion then declined steadily such that 8 days after 'late balloon', less than 20% of papillae were healthy and 2 days later virtually all styles were dark and desiccated.

In 1985 however, at each time interval beyond 'late balloon' that the flowers were examined, those from 2-year-old trees bore styles with a greater proportion of collapsed or 'waterlogged' papillae than did flowers from any other tree age (Fig 6.3.2.1a). Two days later, although

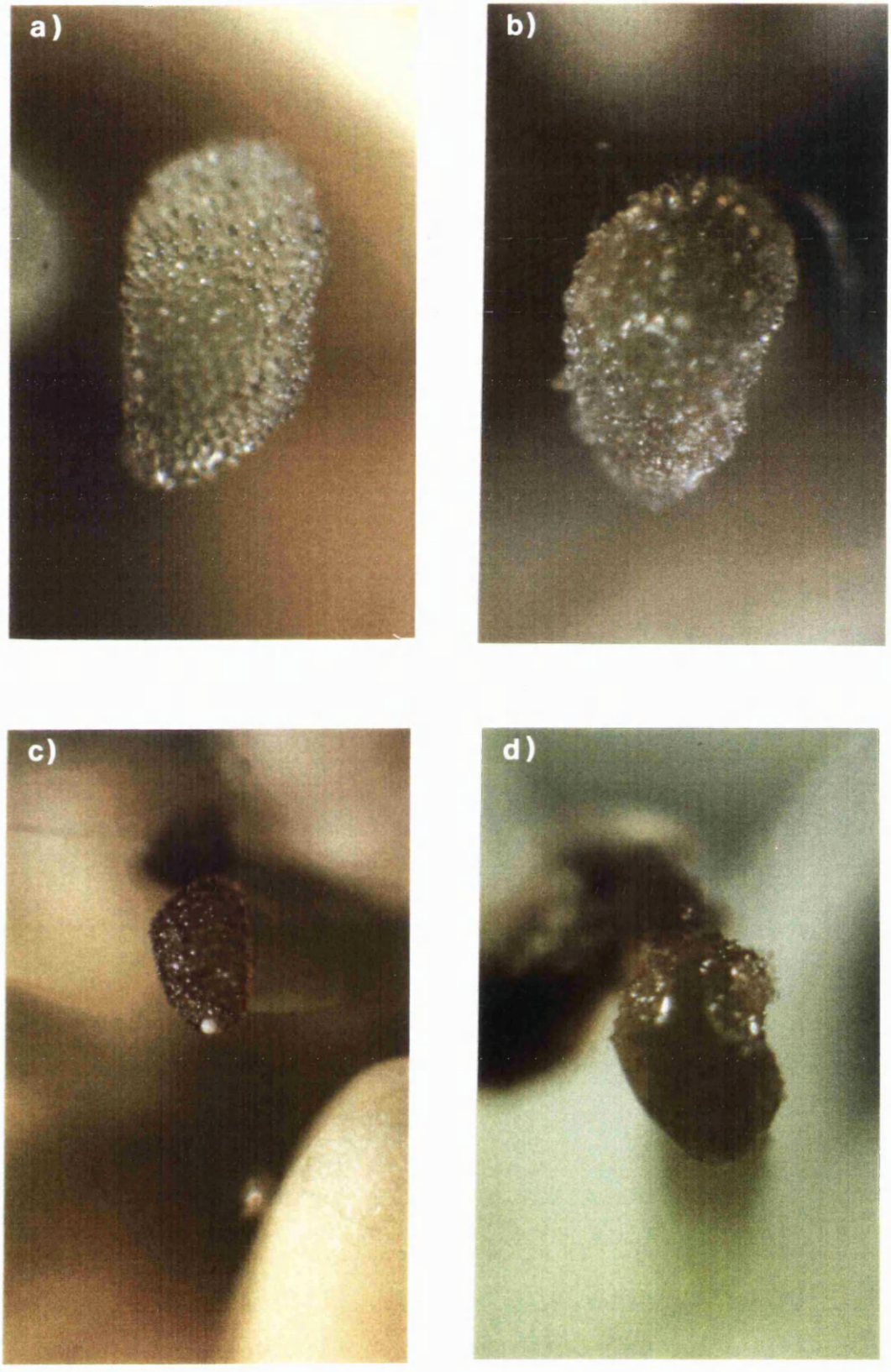
Plate 6.3.2.1 Stigmatic surface in Cox flowers at increasing lengths of time after anthesis.

(a) at anthesis with papillae round and turgid,

(b) some browning of the papillae visible,

(c) more extensive browning and some excess excretions accumulating,

(d) surface almost completely submerged under secretions.


a)
b)
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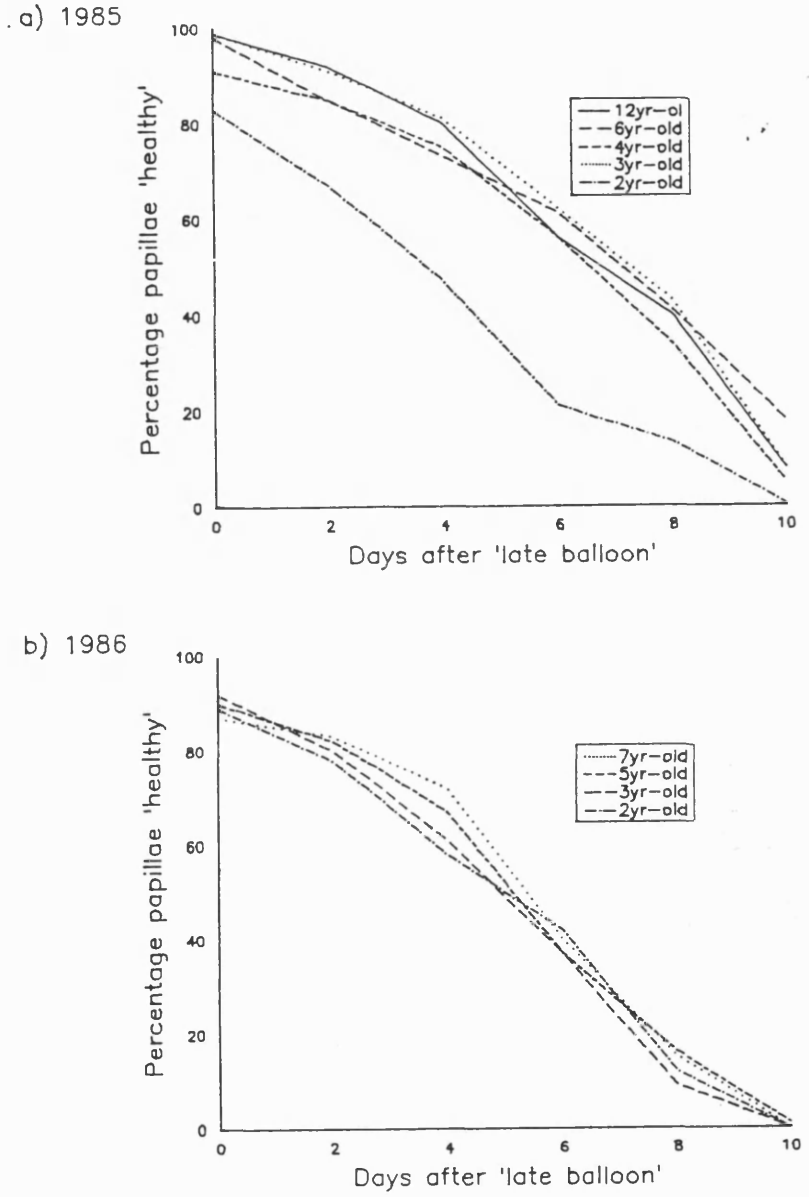


Figures 6.3.2.1a + b
Percentage of stigmatic papillae 'healthy' (turgid, free from browning and excess secretions) at increasing lengths of time after 'late balloon' within flowers from various ages of tree in;

(a) 1985
(b) 1986

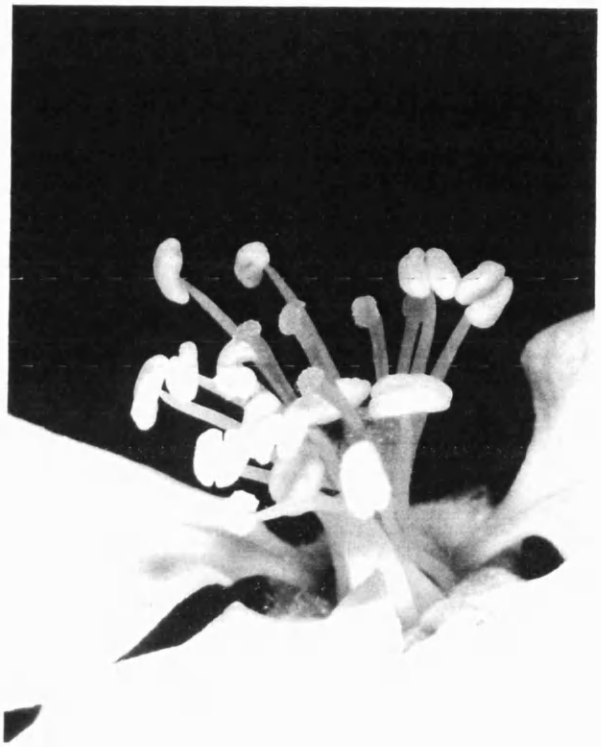
Plate 6.3.2.2 Desiccation of Cox stigmas and styles as observed in 1986.

(a) flower at anthesis, stigmatic papillae and the style both green and healthy,

(b) complete desiccation of stigma

(c) desiccation extending down the style.

a)



b)

c)

styles from the youngest trees still had the lowest proportion of turgid papillae, those from 12-year-old trees had significantly more than did styles from any other tree age.

However, although in both years flowers were harvested at equal intervals of time after 'late balloon', in 1985 this occurred 3 days later on the 2-year-old trees than on the older ones. Consequently, flowers (and styles) from 2-year-old trees developed and flowered within a different time period and climatic environment.

In 1985, the rate of stigmatic degradation over the flowering period of flowers borne on vertical branches was almost identical to that occurring within flowers on the horizontal branches of the same trees (Figure 6.3.2.2a). At 'late balloon' c 93% of papillae appeared healthy and turgid. This declined at c 5%/day for the following 4 days then at 10%/day until less than 10% of papillae appeared healthy at 'late balloon' plus 10 days.

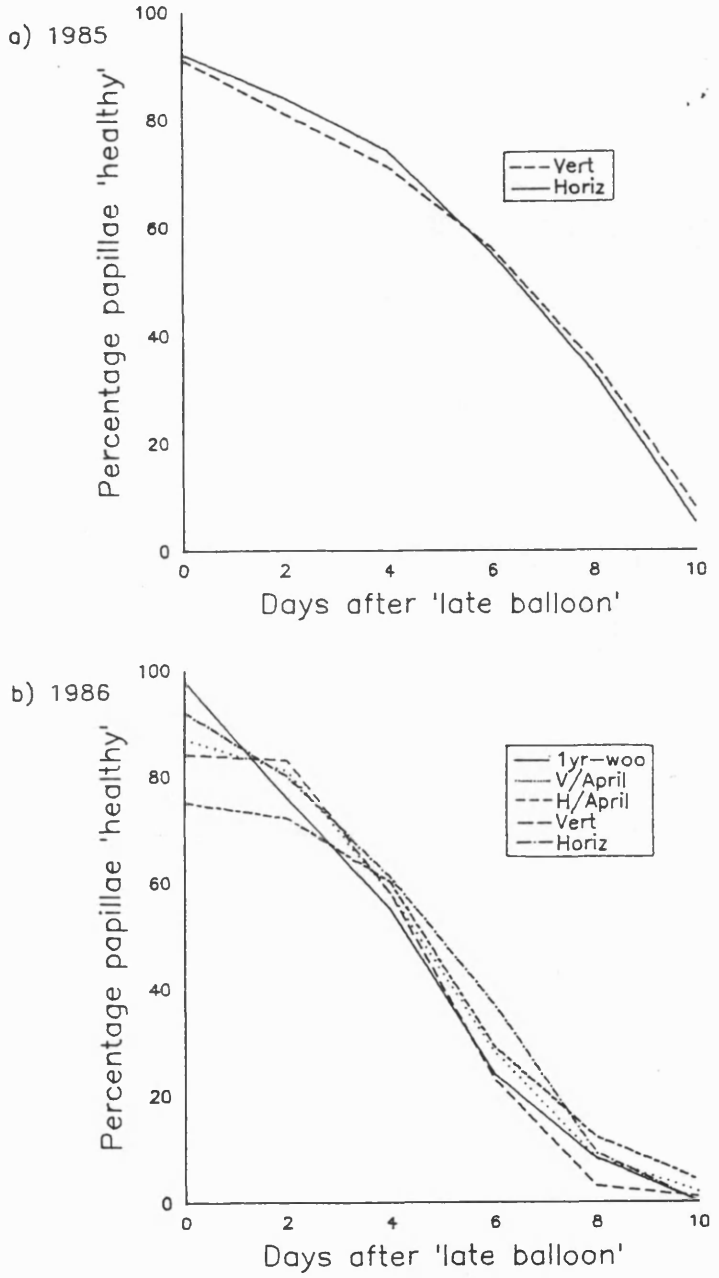
In 1986, of the four branch orientation treatments employed, branches which were vertical prior to flowering but horizontal therafter had flowers with the lowest proportion of turgid papillae when sampled at 'late balloon' (Figure 6.3.2.2b). Flowers from all the other branch orientation treatments had, on average, more than 80% of papillae healthy at this time. Two days after 'late balloon' this difference had been lost and flowers from all branch orientation treatments had similar stigmatic conditions. This situation remained throughout the rest of the flowering period.

At 'late balloon', flowers on 1-year-old wood had a higher proportion of healthy papillae than did those from any other examined situation. However, this was followed by a more rapid decline in condition, such that by 4 days later no differences were apparent between these flowers and those from any other situation.

6.3.3 Pollen Tube Growth

In both years it was seen that although pollen grains germinated freely on the stigma, and many tubes penetrated the style, the numbers reaching the style base prior to harvest 48 or 96 hours later were often very low (Tables 6.3.3.1 and 6.3.3.2). In 1985, 48 hours after pollination few styles from any age of tree had pollen tubes visible at their base but after a further 48 hours incubation more tubes were present at all four assessment sites (Table 6.3.3.1). In 1986, when pollen tube growth assessment was conducted using excised flowers maintained within an incubator, all flowers were fixed 48 hours after pollination. This time period was decided on because the Pollen Tube Index published by Williams (1970b), shows that at a constant temperature of 15°C (as maintained in the incubators), pollen tubes should require 2 days to reach the style base.

One-way analysis of variance using 'tree age' as the factor indicated that in 1985 neither the age of tree upon which flowers were borne, nor branch orientation, had any significant effect on the number of tubes present any point within the style, at either 48 or 96 hours after



Figures 6.3.2.2a + b
Percentage of stigmatic papillae 'healthy' (turgid, free from browning and excess secretions) at increasing lengths of time after 'late balloon' within flowers from different orientations of branch and ages of wood;

(a) 2-year-old wood in horizontal and vertical branches in 1985
(b) i) 2-year-old wood in horizontal branches
ii) 2-year-old wood in vertical branches
iii) 2-year-old wood in branches horizontal until April 3rd, vertical thereafter
iv) 2-year-old wood in branches vertical until April 3rd, horizontal thereafter
v) 1-year-old wood in 1986

Tables 6.3.3.1a + b Number of pollen tubes at various positions down the length of styles from flowers borne on various ages of tree and orientations of branch in 1985 at (a) 48 hours or (b) 96 hours after pollination. All branches are horizontal except where marked 'V' denoting a vertical wood.

a) 48 hours after pollination

age of wood (years)	position down style (see figure 6.2.1.1.)			
V = vertical wood	A	B	C	D
2	16·4	7·3	5·2	1·7
3	15·6	9·3	1·1	0·3
4	13·5	6·7	2·2	1·4
4V	15·6	6·3	1·7	1·0
6	16·8	8·3	1·5	0·3
12	18·3	7·5	3·2	0·5
S.E.D.	2·4	1·9	1·2	0·4

(b) 96 hours after pollination

age of wood (years)	position down style (see figure 6.2.1.1.)			
V = vertical wood	A	B	C	D
2	21·2	13·3	8·3	3·4
3	24·6	17·4	9·7	1·8
4	23·3	15·1	6·4	3·4
4V	25·3	16·4	6·1	2·6
6	26·4	18·8	7·6	2·9
12	21·9	15·3	8·1	3·6
S.E.D.	2·7	1·8	1·4	1·2

Tables 6.3.3.2a-d Number of pollen tubes visible at various positions down the styles (see figure 6.2.1.1.) of flowers from various ages of tree when pollinated at increasing lengths of time after late balloon in 1986. Mean values bearing the same letter are not significantly different at $P \leqslant 0.05$.

(a) position A

time of pollination	tree age (years)				
(days after late balloon)	2	3	4	5	date mean
0	23·3	25·3	27·7	28·4	26·2a
2	14·5	12·7	17·5	19·8	16·1b
4	5·7	10·7	10·5	10·0	9·2c
6	0·4	6·1	3·1	2·8	3·1d
8	3·9	1·1	1·9	3·1	3·9d
age mean	9·6a	11·2ab	10·1a	12·8b	

S.E.D.: Days × Age = 1·997; Age = 1·153; Days = 0·999.

(b) position B

time of pollination	tree age (years)				
(days after late balloon)	2	3	4	5	date mean
0	16·2	13·5	18·3	18·4	16·6a
2	8·2	4·1	12·5	8·2	6·6b
4	2·6	5·5	5·9	5·8	4·9c
6	0·6	1·8	1·1	0·9	1·1d
8	0·6	0·9	0·8	1·7	1·0d
age mean	5·6a	5·2a	7·7b	7·0b	

S.E.D.: Days × Age = 1·090; Age = 0·629; Days = 0·545.

(c) position C

time of pollination	tree age (years)				
(days after late balloon)	2	3	4	5	date mean
0	11·7	10·2	13·4	13·8	12·3a
2	5·1	2·1	9·7	6·7	5·9b
4	2·2	4·5	5·0	3·1	3·6c
6	0·0	0·7	0·6	0·7	0·5d
8	0·5	0·2	0·7	0·7	0·5d
age mean	3·9ab	3.5a	5·9b	5·0b	

S.E.D.: Days × Age = 0·944; Age = 0·545; Days = 0·472.

(d) position D

time of pollination	tree age (years)				
(days after late balloon)	2	3	4	5	date mean
0	4·2	6·2	6·5	7·1	6·0a
2	4·1	1·3	7·4	5·0	4·5b
4	1·7	3·8	3·1	2·5	2·8c
6	0·4	0·5	0·4	0·5	0·5d
8	0·3	0·1	0·3	0·0	0·2d
age mean	2·1a	2·4ab	3·5b	3·0b	

S.E.D.: Days × Age = 0·688; Age = 0·397; Days = 0·344.

pollination (Table 6.3.3.1). Although, 48 hours after pollination, an average of 16.4 pollen tubes were visible at point A, very few had reached the style base. After a further 48 hours the average number of tubes at point A had increased to 23.7, and average of 3.0 had reached the style bases.

The pattern of pollen tube growth within flowers borne on vertical branches was very similar to that of flowers on horizontal branches within the same trees.

In 1986, tube growth following pollination at increasing lengths of time after 'late balloon', again indicated that tree age had little influence (Table 6.3.3.2). Although large variations were found in the number of tubes present, within all tree ages pollen tube growth was highly affected by time of pollination. The maximum numbers of tubes penetrated the style when pollination occurred at anthesis (23-28 tubes at Region A, 4-6 reaching the style base) but pollination only 2 days later resulted in a 50% decline in the number of tubes present at all assessment sites. When pollination was conducted 4, or 6 days after 'late balloon', the number of tubes present in all regions were consistently lower than resulted from previous pollinations and flowers on 2-year-old trees had fewer pollen tubes visible at all assessment sites than did those from any other tree age. Pollinations occurring 8 or 10 days after 'late balloon' gave rise to no apparent differences in pollen tube growth between the flowers from the different ages of tree; all had very few tubes visible at any point.

Within all tree ages, when pollination was delayed more than 4 days after 'late balloon', few pollen tubes were seen to penetrate any styles, the average number of tubes reaching the style base being less than one. This suggests that following pollinations at these times not even one ovule per flower could possibly have been fertilised.

Within the 5-year-old trees, flowers from different orientations and ages of wood displayed few differences in the rate, or number of growing pollen tubes over the flowering period (Table 6.3.3.3). In all situations, most tubes (26.8 on average) penetrated the style when pollination occurred at 'late balloon', c 25% of these reaching the style base 48 hours later. Pollination 2 days later reduced the number of penetrating tubes to almost half that occurring at 'late ballon' but c 33% of these reached the style base within 48 hours. Pollination 6, or 8 days after 'late ballon' gave rise to very few tubes penetrating or growing through any styles.

6.3.4 Ovule Degradation

The ovules of Cox are longitudinally orientated in bi-partite locules with one ovule on each side of the locule. They are anatropous, crassinucellary and have two integuments. The embryo sac is relatively large, elongate along the ovule axis and is monosporic and eight nucleate of the *Polygonum* type.

Nuclei of the egg sac and synergids, the large central cell (with its polar nuclei either separate or in some stage of fusion), and occasionally the antipodal cells were visible when ovules

Tables 6.3.3.3a-d Number of pollen tubes present at various positions down the style (see figure 6.2.1.1.) of flowers borne within different branch orientation treatments or ages of wood (as described below) when pollinated at increasing lengths of time after late balloon. Mean values bearing the same letter are not significantly different at $P \leqslant 0.05$. Treatments were:-

H = 2-year-old horizontal wood
V = 2-year-old vertical wood
Ha = 2-year-old wood vertical prior to flowering, horizontal thereafter
Va = 2-year-old wood horizontal prior to flowering, vertical thereafter
Hi = 1-year-old horizontal wood

(a) position A

time of pollination	branch orientation/wood age					
days after late balloon	H	V	Ha	Va	Hi	date mean
0	27·8	28·2	23·4	26·3	28·3	26·8d
2	17·5	14·9	15·6	16·3	14·2	15·7c
4	10·8	15·7	10·4	11·2	9·3	8·3b
6	2·2	2·7	4·3	3·1	2·9	3·0a
8	1·9	1·2	1·6	3·1	2·1	2·0a
treatment mean	12·0a	12·5a	11·1a	12·0a	11·2a	

S.E.D.: Treatments × Days = 2·234; Treatments = 1·436; Days = 1·043.

(b) position B

time of pollination	branch orientation/wood age					
days after late balloon	H	V	Ha	Va	Hi	date mean
0	18·3	13·0	16·9	14·1	16·7	15·8d
2	12·5	10·4	9·7	12·1	9·5	10·8c
4	5·9	6·2	6·1	8·6	3·9	6·1b
6	1·1	2·0	2·6	2·3	1·8	2·0a
8	0·8	0·7	0·9	1·3	1·2	1·0a
treatment mean	7·7a	6·5a	7·2a	7·7a	6·6a	

S.E.D.: Treatments × Days = 2·141; Treatments = 0·893; Days = 0·632.

(c) position C

time of pollination	branch orientation/wood age					
days after late balloon	H	V	Ha	Va	Hi	date mean
0	13·4	10·6	9·6	11·2	12·4	11·4d
2	9·7	6·6	7·6	8·7	7·3	8·0c
4	4·9	5·2	4·8	4·8	3·0	4·5b
6	0·6	1·8	1·7	1·4	1·1	1·3a
8	0·7	0·5	0·6	0·9	0·3	0·6a
treatment mean	5·9c	4·9bc	4·8b	3·4a	4·8b	

S.E.D.: Treatments × Days = 0·973; Treatments = 0·457; Days = 0·454.

(d) position D

time of pollination	branch orientation/wood age					
days after late balloon	H	V	Ha	Va	Hi	date mean
0	6·3	4·5	7·3	4·6	5·2	5·6c
2	7·4	6·9	4·4	6·9	6·6	6·4c
4	3·0	3·4	4·3	2·9	4·2	3·6b
6	0·4	1·0	1·1	0·9	0·7	0·8a
8	0·3	0·4	0·1	0·7	0·1	0·3a
treatment mean	3·5a	3·2a	3·4a	3·2a	3·4a	

S.E.D.: Treatments × Days = 0·103; Treatments = 0·621; Days = 0·539.

were examined microscopically. Because the wax sections were approximately 10μm thick, all components of the entire ovule were not usually observed together in a single section but more often within several consecutive ones.

In 1985, examination and classification of ovules within unpollinated flowers collected at 'late balloon' from 12-year-old trees showed 66% of examined ovules to be 'healthy', 24% immature, and 12% overmature (Table 6.3.4.1). Of the flowers collected from the other ages of tree, as tree age decreased there was a general decrease in the proportion of ovules which appeared healthy (Table 6.3.4.1). In 2-year-old trees, only 17% of ovules were mature and healthy at this time, 52% were immature, and 29% (the highest proportion within flowers from any tree age) were overmature. Contingency tables indicated that when compared to flowers from older trees, significantly more ($P \leqslant 0.01$) ovules within flowers from the youngest trees were immature at 'late balloon', and that significantly more were also degenerate. This meant that a much lower proportion of ovules were mature and healthy at this time in these flowers than in those from other ages ($P \leqslant 0.001$). Flowers from vertical branches within the 4-year-old trees were slightly, but not significantly less mature than were those from the horizontal branches.

Using the Pollen Tube Index (Williams 1970b) it was calculated that in 1985, it would require 7 days from pollination for pollen tubes to grow through the style and reach the ovule. Therefore it is the condition of the ovule at this time, rather than at anthesis, which determines whether fertilisation could be successful. Therefore, flowers harvested eight days after 'late balloon' were sectioned and examined as before.

It was seen that flowers from the youngest trees contained the lowest proportion of healthy ovules and those from the 6-year-old trees the highest (Table 6.3.4.1). 58% of ovules within flowers from 6-year-old trees were healthy, 28% were overmature, and 10% remained immature. Within flowers from the youngest trees, only 26% of ovules appeared healthy, 36% were overmature but the largest proportion, (38%) remained immature. Flowers from vertical branches were again similar to those from horizontal branches within the same trees, having 41% of ovules mature, 40% degenerate, and 19% immature.

It is possible that the physiological condition of an embryo sac and any time induced changes within it may be influenced by whether or not pollination has occurred. Thus although embryo sacs of unpollinated (but not emasculated) flowers were examined in 1985, they were examined again in 1986, this time within flowers which had been pollinated at one of several predetermined times after 'late balloon'. Pollinations were conducted in the field, in parallel with those of the 1986 EPP experiment (Section 5.2.4), and took place either at 'late balloon' or 2, 4, 6, 8 or 10 days after this. Primary interest concerned the condition of the ovule at the time when a pollen tube might reach it rather than any time prior to this, therefore in 1986 flowers were harvested 4 days after pollination and fixed and stained as in 1985.

Tables 6.3.4.1a + b Percentage of ovules immature, mature or degenerate in flowers from different ages of tree. Harvested at (a) late balloon or 8 days later (1985) and (b) at late balloon or 4 or 8 days later (1986). Flowers were all taken from horizontal 2-year-old wood except when indicated with a 'V' in which case branches were vertical. Approximately 60 ovules were examined in each situation. Data was analysed using contingency tables.

(a) 1985

time of harvest (days after late balloon)	age of tree (V = vert. wood)	condition of ovule immature	mature	degenerate
	2	52	17	29
	3	40	44	16
0	4	47	41	11
	4V	50	38	12
	6	27	53	19
	12	24	64	12
$\chi^2 = 61 \cdot 45^{***}$				
	2	38	26	36
	3	31	44	25
8	4	20	44	36
	4V	19	41	40
	6	14	58	28
	12	15	49	36
$\chi^2 = 37 \cdot 65^{***}$				

(b) 1986

time of harvest (days after late balloon)	age of tree (yrs.) (V = vert. wood)	ovule condition immature	mature	degenerate
	2	15	47	38
0	3	21	75	4
	5	8	87	5
	7	3	94	3
$\chi^2 = 102 \cdot 40^{***}$				
	2	0	0	100
4	3	8	22	70
	5	10	67	23
	7	0	55	45
$\chi^2 = 151 \cdot 96^{***}$				
	2	0	0	100
8	3	0	0	100
	5	0	0	100
	7	0	0	100

It was seen that as in 1985, the proportion of ovules in different stages of development varied according to both tree age and pollination/harvesting date (Table 6.3.4.1b). Of flowers pollinated at 'late balloon', those from the oldest (7-year-old) trees had the highest proportion (94%) of healthy ovules when harvested 4 days later; those from the youngest (2-year-old) trees had the lowest (45%). In these young trees a large proportion (38%), of ovules within flowers pollinated at 'late ballon' were already showing signs of degeneration compared to c 4% of those within flowers from the older trees. Contingency tables showed these differences to be significant at $P \leqslant 0.001$.

When pollination was delayed until 4 days after 'late balloon', tree-age differences became more pronounced and were again significant at $P \leqslant 0.001$. Four days later all of the ovules within flowers from the youngest trees, and 70% of those from the 3-year-old trees, were degenerate. Flowers from the 5-year-old trees had the highest proportion of healthy ovules (67%) at this time, followed by those from the 7-year-old trees (55%).

However, these figures obscure differences in the degree of degradation apparent within 'over-mature' ovules. Within those from the older trees the degradation observed was in the form of a general disorganisation of the embryo sac contents occasionally associated with some nucellar shrinkage. Within flowers from the younger trees ovule degradation was more advanced and extreme. Often the embryo sac was not visible at all, the nucellus and integuments having collapsed completely.

Within all ages of tree, flowers pollinated 8 days after 'late balloon' displayed extreme ovular degradation when harvested 4 days later. All ovules examined were collapsed and shrunken.

Of the flowers borne on the different branch orientation treatments, all had a similar number of apparently healthy ovules present 4 days after pollination at 'late balloon' (Table 6.3.4.2). This ranged between 77% in flowers borne on vertical branches to 94% in those borne on horizontal branches. Few degenerate ovules were apparent in these flowers, and of those that were, degeneration was largely confined to the egg sac and the nucellus tip. Although flowers borne on 'horizontal branch' treatments had few immature ovules, 21% and 14% of ovules were immature in flowers from 'continuously vertical' or 'vertical during flowering' treatments respectively. Contingency tables showed the former to be significant at $P \leqslant 0.01$.

Examination of flowers pollinated 4 days after 'late balloon' showed various differences to exist. Generally, many more ovules were now degenerate compared to those flowers pollinated earlier, and degeneration was often more extreme and widespread. Flowers borne on the two 'vertical branch' treatments had only c 50% of ovules still healthy compared to 67% and 72% within flowers from the horizontal branch treatments (Table 6.3.4.2).

At both times of sampling flowers from 1-year-old wood had fewer healthy ovules than did those from older wood. Only 56% were mature and healthy four days after pollination at 'late

Table 6.3.4.2. Percentage of ovules immature, mature or degenerate in flowers from various branch orientation treatments (described below) at 4 days after pollination at either late balloon or late balloon plus 4 days.
Treatments were:-

H = branches horizontal prior to and during flowering
V = branches vertical prior to and during flowering
Ha = branches vertical prior to flowering, horizontal thereafter
Va = branches horizontal prior to flowering, vertical thereafter
Hi = 1-year-old horizontal wood

time of pollination (days after late balloon)	branch orientation/age	condition of ovule immature	mature	degenerate
	H	8	87	5
	Ha	0	94	6
0	V	21	77	2
	Va	14	82	4
	Hi	17	56	27
$\chi^2 = 81 \cdot 04^{***}$				
	H	10	67	23
	Ha	3	72	25
4	V	6	52	42
	Va	3	56	41
	Hi	0	21	79
$\chi^2 = 90 \cdot 74^{***}$				

Table 6.3.4.3. Percentage of ovules displaying fluorescence (number of ovules examined) when observed under ultraviolet light. Ovules were fixed 2 days after pollination which occurred at increasing lengths of time after late balloon. Data (for days 4, 6 and 8 only) were analysed using contingency tables.

time of pollination (days after late balloon)	age of tree (years) 2	3	5	7
0	8	0	0	0
	(50)	(47)	(48)	(50)
2	1 · 5	0	0	0
	(70)	(80)	(80)	(80)
4	27	22	0	2
	(60)	(56)	(70)	(54)
6	93	53	29	27
	(60)	(60)	(56)	(60)
8	100	100	98	100
	(39)	(40)	(55)	(50)
10	100	100	100	100
	(50)	(56)	(50)	(48)
$\chi^2 = 73 \cdot 43^{***}$				

balloon', and within flowers pollinated later, only 21% were. This reduced number of healthy ovules was almost entirely due to an increased number being degenerate rather than to embryo sac immaturity (Table 6.3.4.2).

Thus it would appear that in 1985 flowers collected 8 days after 'full bloom' generally had a reasonable proportion of healthy ovules, and some apparently immature ovules perhaps continuing to develop towards maturity. In 1986 however, flowers collected at the same length of time after anthesis, having been pollinated 4 days previously generally had few ovules still immature; those from the younger trees displaying extreme levels of degeneration in all ovules examined.

In recent years fluorescence microscopy has been used to assess ovule condition in cherry (Anvari and Stosser 1978) but at present is rarely used for pome fruit. The speed and ease of such a method in comparison to traditional sectioning makes it an attractive option if a reliable technique were available. Consequently material additional to this series of experiments was used to conduct preliminary work comparing and assessing both the fluorescent and sectioning techniques.

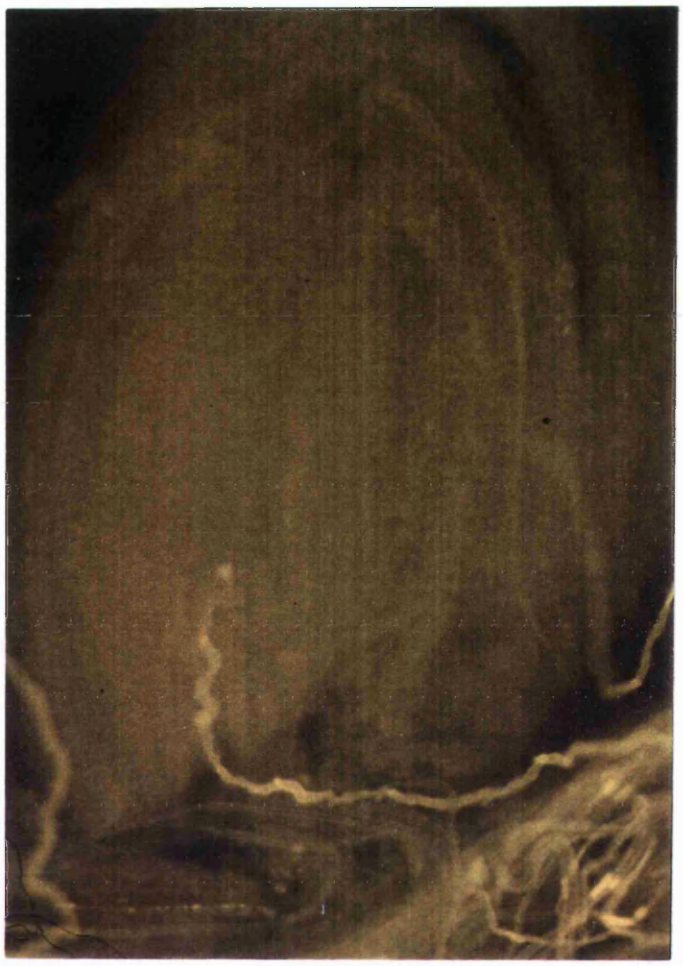
During the 1986 assessment of pollen tube growth described in Section 6.3.3, flowers from different ages of tree were collected at, and at 2 day intervals after 'late balloon', hand pollinated, then maintained in damp 'Oasis' within an incubator at 15°C for 2 days prior to fixing the stylar and receptacular material in sodium sulphite. When preparing styles for pollen tube counts, after rinsing in distilled water and soaking in 0.1M K_3PO_4 excess receptacle material was pared away from the ovaries such that styles could be extracted. Ovaries were left attached to individual styles and squashed lightly under a cover slip prior to microscopic examination under ultraviolet light. Degenerate ovules are reported to display fluorescence under such conditions (Anvari and Stosser 1978). Generally ovules from flowers harvested four days after pollination at 'late balloon' or 'late balloon' plus 2 days, did not fluoresce (Table 6.3.4.3) but of those pollinated and harvested later than this, some fluorescence was visible in several. This fluorescence generally appeared first at the chalazal end of ovules, then became more diffuse with occasional concentration within the egg sac itself (Plate 6.3.4.1.). In ovules of young trees it appeared earlier, ('late balloon' plus six days) and affected a higher proportion of ovules than in ovules from older trees (first appearance of fluorescence at 'late balloon' plus 8 days). Thus if fluorescence is indicative of degenerative ovules then the two methods are in good agreement. However, there was a delay of approximately 4 days between the first signs of degeneration as seen by sectioning and the appearance of a fluorescent reaction. The flourescent reaction indicates the presence of β-glucan, which is probably released as a result of the general breakdown of ovular tissue, which may occur only after embryo sac degeneration has taken place.

Plate 6.3.4.1 Ovules of Cox flowers as seen under ultraviolet light.

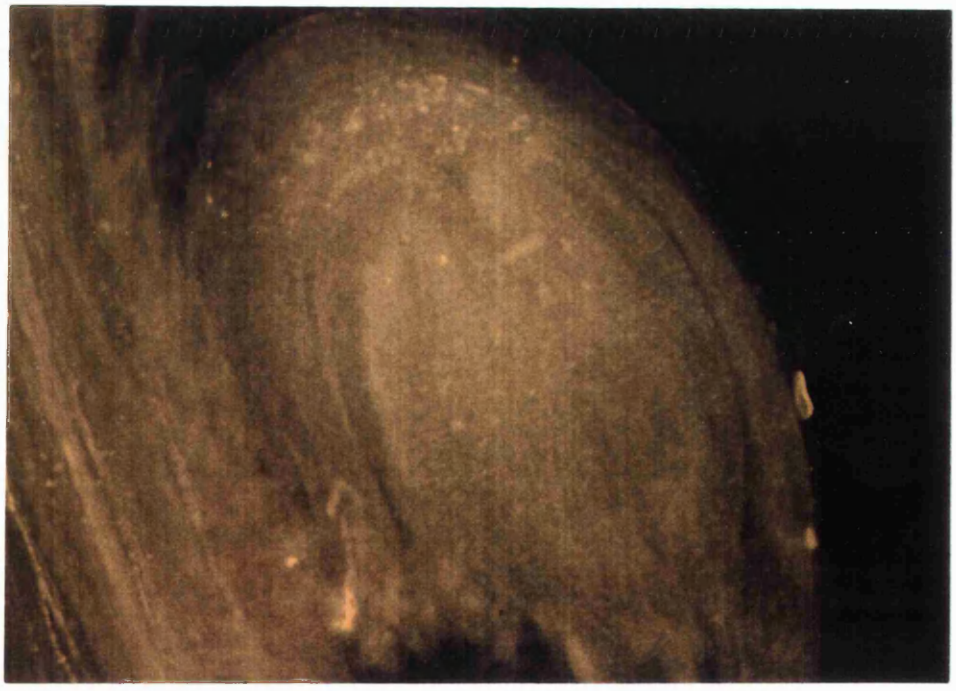
(a) ovule with no sign of fluorescence and a pollen tube penetrating into the egg-sac,

(b) ovule with fluoresence at its chalazal end - although pollen tubes are visible in the style, none were ever seen to penetrate an ovule showing fluorescence.

a)



b)



In conclusion, it seems possible that this fluorescence technique is indicative of ovular condition but care must be taken with the experimental design to consider the delayed response.

6.3.5 Embryo development

Within the various ages of tree examined, a wide range of embryo conditions was observed. These included apparently unfertilised ovules, fertilised ovules showing good endosperm and embryo development, and fertilised ovules with indications of subsequent degeneration (Plate 6.3.5.1). Of the unfertilised ovules, a few of these appeared completely healthy but most were completely degenerate, the nucellus and integuments having collapsed completely.

Flowers from the older (5- and 7-year-old) trees, pollinated at 'late balloon' and harvested 14 days later had many well developed embryos present (Table 6.3.5.1). These displayed a much elongated embryo sac, having lengthened from c 90 μm in unpollinated ovules to c 575 μm and often extending throughout the entire length of the nucellus. Within these, many free endosperm nuclei were visible. The embryo itself usually had a suspensor of 3-4 cells (although occasionally up to 10 were seen), a basal cell and the embryo proper. Within these groups, the majority of embryo sacs appeared healthy: all had been fertilised and had undergone initial development (Table 6.3.5.1). Occasionally the embryo sac contents appeared slightly disorganised and more densely stained, suggesting some degeneration and imminent abortion. Within individual flowers, embryo development varied, sometimes with both well developed and degenerating embryos sharing the same locule.

Within flowers from the youngest trees, no developing embryos were seen at all. All embryo sacs were completely collapsed and shrunken, often with complete degeneration of the nucellus and integuments as well. None of these showed any signs that fertilisation had been achieved.

The condition of flowers from the 3-year-old trees was intermediate to this; most ovules had been fertilised and some embryo development had occurred, though some had degenerated prior to fertilisation while others showed signs of degeneration subsequent to fertilisation.

In the flowers pollinated 2 days after 'late balloon', fewer healthy embryos were present. Again none were found in flowers from the youngest trees, and only in 18% of examined ovules from the 3-year-old trees. Within flowers from the older trees, less than 50% of ovules had healthy, developing embryos; the majority had been fertilised but subsequently degenerated.

Following pollination at 4 days after 'late balloon', no flowers/developing fruit were present on the 2-year-old trees 14 days later. Although fruitlets still remained on the older trees at this time, their external appearance indicated lack of growth and very few healthy embryos were found. The majority of ovules had not been fertilised, and of those which had, most showed signs of degeneration.

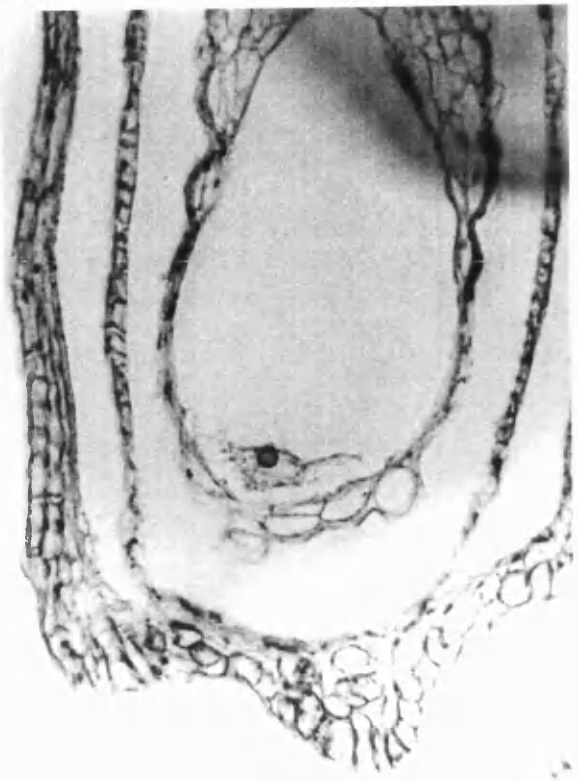
Plate 6.3.5.1 Variety of ovular conditions found 14 days after pollination.

(a) unfertilised ovule,

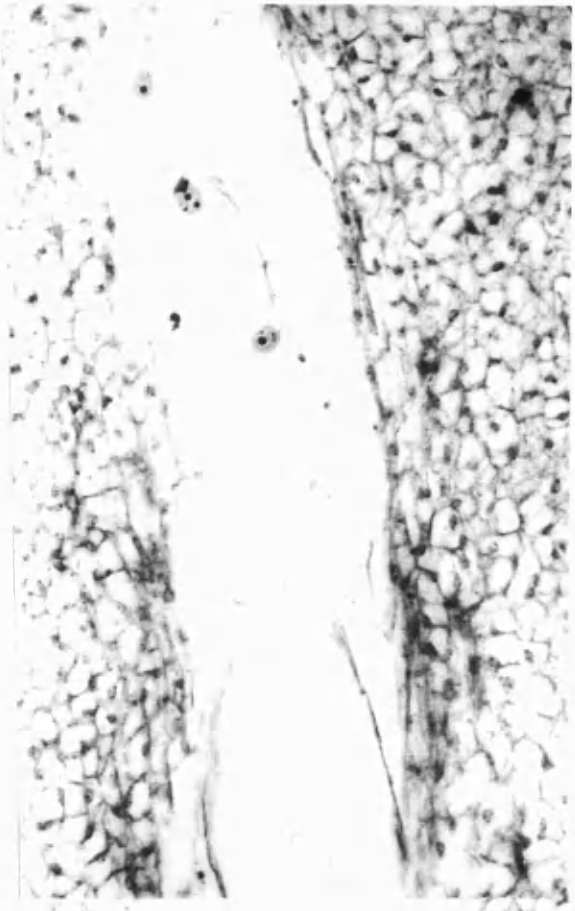
(b) fertilised ovule showing good endosperm and embryo development,

(c) fertilised ovule which has subsequently degenerated.

a)



b)



c)

Table 6.3.5.1. Percentage of examined embryos which were either unfertilised, fertilised and healthy, or fertilised but degenerate within flowers from various ages of tree pollinated at 0, 2 or 4 days after late balloon.

time of pollination (days after late balloon)	age of tree (years)	condition of embryo		
		unfertilised	fertilised (healthy)	fertilised (degenerate)
	2	100	0	0
0	3	12	80	8
	5	6	84	12
	7	8	82	10
$\chi^2 = 293 \cdot 21^{***}$				
	2	100	0	0
2	3	42	18	40
	5	0	47	53
	7	4	38	60
$\chi^2 = 285 \cdot 04^{***}$				
	2	[1]	[1]	[1]
4	3	92	0	8
	5	61	4	35
	7	65	4	31
$\chi^2 = 29 \cdot 04^{**}$				

[1] All flowers abcised prior to sample collection.

Virtually no fruitlets from pollinations later than 4 days after 'late balloon' were present 14 days later and no microscopic examination was made of these.

6.4 Discussion.

6.4.1 Flower size.

In both years of observation, flowers from the younger trees had shorter pedicels and smaller receptacles than did those from older trees. These parameters were also smaller in axillary flowers compared to flowers from older wood. Because flowers on young trees and axillary flowers both set very badly (Chapter 5) it might be suggested that decreased flower size is associated with depressed setting ability. This would be in line with the general feeling that small flowers are 'weak' and set badly, a situation which has been described by many workers. Goldwin (1978) visually categorised flower clusters as being 'weak' or 'strong' depending on their size, and Ferree and Rom (1984) described 'weak' spurs as bearing flowers which were small and set badly. Similarly Williams (1965) described high 'quality' flowers as being larger and more vigorous and Hill-Cottingham and Williams (1967) described 'strong', well setting flowers achieved by late summer nitrogen application as being large.

In agreement with this, by manipulating spring temperature, Abbott (1971) induced the formation of small flowers with a poor setting ability to be formed. Similarly Buszard (1983) found flowers from previously heavily cropped trees to be smaller and have reduced setting ability compared to those from defruited trees. Also, in naval orange, Guardioli *et al* (1984) found that conditions which increased ovary weight (and presumably, size) also increased fruit set potential.

However, other work has found no relationship between flower size and 'quality'. Miller (1988), by manipulation of spring temperature obtained flowers of very different 'qualities' but found no difference in receptacle diameter or pedicel length. In contrast she found 'weak' flowers to have larger and heavier petals than did 'stronger' flowers and therefore to have the appearance of being larger overall.

Although results obtained here generally agree with the premise that poorly setting flowers are small, one contradiction stands out. In 1986, flowers from 7-year-old trees had pedicels equivalent in length to those of the youngest trees and significantly shorter than on flowers from 5-year-old trees even though fruit set of the two older tree ages was equivalent and 4 times greater than that on the youngest trees.

Thus overall it would appear that flower size can be an indicator of 'quality' but that it does not always hold true.

6.4.2 Stigmatic surfaces and pollen tube growth.

a) Age of tree

In 1985, although the rate at which stigmatic surfaces degenerated was the same within flowers from all ages of tree, those from 2-year-old trees had a lower proportion of 'healthy' papillae present at every sampling time. In this year pollen tube growth was only assessed following pollination at anthesis; no 'between tree-age' differences in the number of tubes present at various distances down the style were seen.

This suggests that although at the time of pollination, flowers from 2-year-old trees had a lower proportion of 'healthy' papillae than did those from older trees, this did not adversely affect the number of pollen tubes able to penetrate and grow through the style.

In 1986, as in 1985, the proportion of 'healthy' papillae within flowers from all tree ages decreased as time beyond 'late balloon' increased. Unlike 1985, there were no differences between flowers from the different ages of tree. Pollination at each sampling time showed that the number of pollen tubes penetrating the styles decreased as time after 'late balloon' increased. This contradicts the findings of Braun and Stosser (1985) who reported that although the papillae of three different apple cultivars were only turgid for one or two days after anthesis, pollen germination and subsequent pollen tube growth remained unaffected even when pollination occurred twelve days after anthesis. Similarly Stosser and Anvari (1983) found that although the papillae of 'sweet cherry' styles had collapsed, pollen could still germinate on a stigma 10 days after anthesis. They therefore concluded that the age of the stigma and the condition of the papillae did not substantially affect pollen tube growth. It is interesting to note here that Herrero (1983) stated that in pear turgid papillae were associated with unreceptive stigmas and that 'the stigmatic surface that supports pollen germination offers the typical disorganised appearance of receptive stigmas'.

To ease examination of data regarding the quality of stigmatic surfaces, and pollen tube growth in relation to one another, how they both changed with time was plotted on the same graph after first being put on an arbitrary scale. Two things were intended. One was to investigate if there was any relationship between the number of tubes penetrating the stigma and the latters observed surface condition. The second was to see how pollen tube growth through the length of the style was affected by any time induced changes within it.

To this end; firstly the number of tubes penetrating the stigma after pollination at 'late balloon' (when the majority of papillae were turgiid), on each age of tree, was given the score 100. Then, for each subsequent pollination date, the number of tubes penetrating the stigma was expressed as a percentage of the number which had penetrated on Day 1 (red line on Figure 6.4.2.1). This was plotted against the score for stigmatic condition on each date.

This showed that, on all ages of tree, the rate at which the number of pollen tubes penetrating and growing through the style decreased, as time between anthesis and pollination in-

creased, was greater than the rate at which stigmatic surfaces degenerated (Figure 6.4.2.1). This might suggest either that the stigmatic surface need only degenerate slightly before inhibiting pollen tube penetration, or else that some other factor is involved in this inhibition.

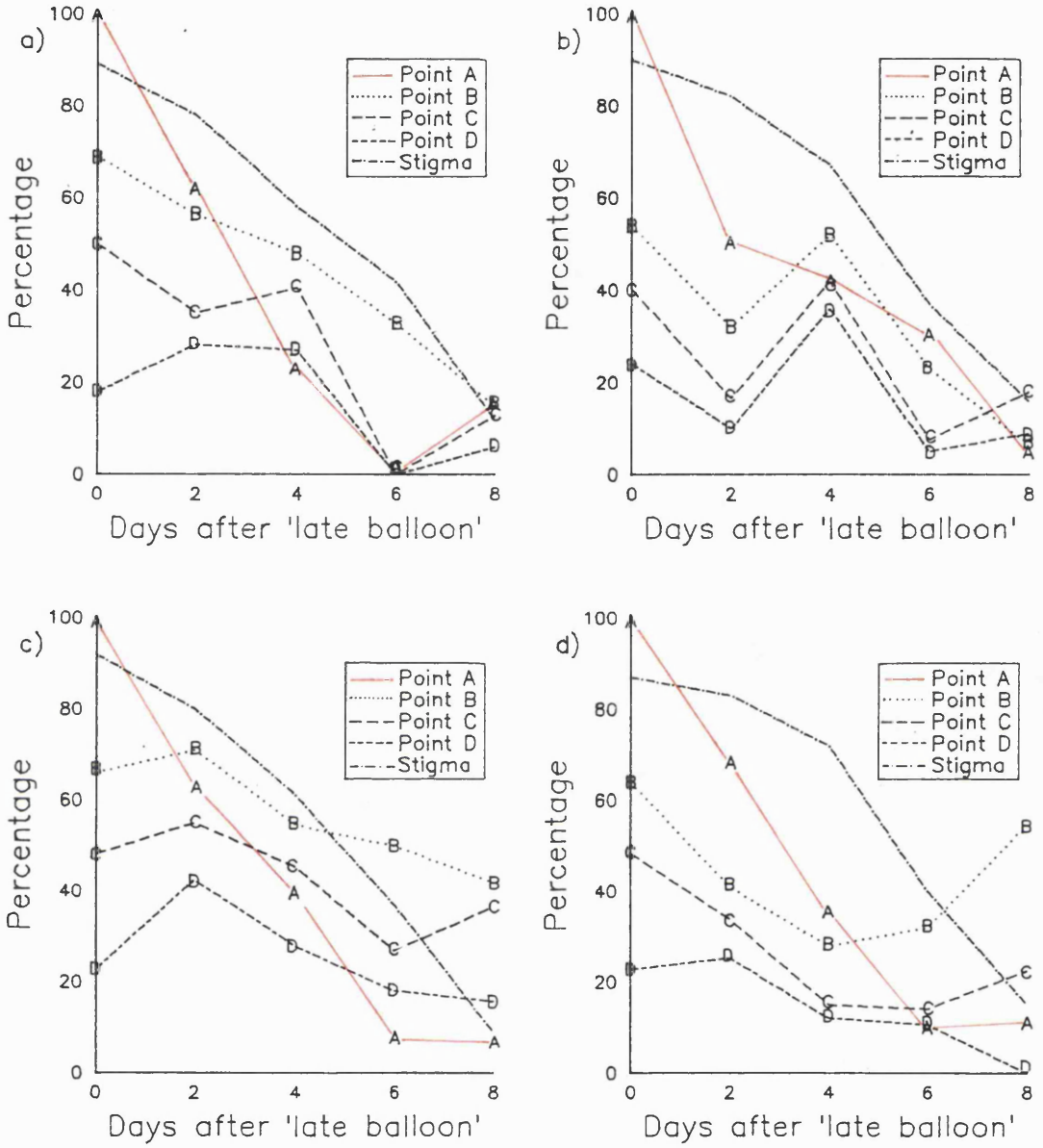
In a study on starch accumulation within stylar transmitting tissue of cherries Stosser and Neubeller (1980) found that this was maximal at anthesis and degenerated within a few days. If this starch is used to provide nourishment for growing pollen tubes (as suggested by Stosser and Anvari (1983)), then its degradation might perhaps limit either the number of tubes able to grow or their rate of growth. However, if stylar breakdown is important, then it might be expected to exert an increasing effect as distance down the style increases. Therefore the percentage of tubes which having penetrated the stigma, subsequently grew down through the style was also calculated for each pollination date. This is also shown on Figure 6.4.2.1 and suggests that in some cases pollen tube growth through the style itself, rather than just the initial penetration of the stigma, may be inhibited as time beyond anthesis increases. In the 2-year-old trees particularly, as time beyond anthesis increased, there was a distinct decrease in the proportion of tubes which having penetrated the stigma subsequently grew through the style (Figure 6.4.2.1a). In the 5-year-old trees though, this was much less apparent and there was only a very slight decline in the percentage which grew through the style throughout the assessment period. Flowers from 3- and 7-year-old trees were intermediate in this.

Thus the situation within the 5-year-old trees supports the results of Braun and Stosser (1985) who found that in the apple varieties studied, breakdown of stylar transmitting tissue did not influence pollen tube growth. However, results for the other ages of tree suggest that some factor within the style itself was inhibiting tube penetration and thus in certain circumstances stylar degeneration may be an important contributor to poor pollen tube penetration.

It therefore appears that the reduction in number of tubes growing through the styles of flowers on 5-year-old trees, as pollination was increasingly delayed after 'late balloon', was due to mainly to an inhibition of pollen germination and/or initial stylar penetration rather than growth *per se*; but that within the flowers on the other ages of tree pollen tube penetration was inhibited in both stages.

b) Orientation and Age of wood effects.

In both years of examination there were no major effects of branch orientation on either the condition of the stigmatic surafce or the numbers of pollen tubes penetrating the style at any time during the flowering period. The only point of note was that flowers from branches which were vertical prior to flowering but horizontal thereafter had, at 'late balloon', a lower proportion of turgid papillae. This did not however appear to exert any influence on the number of pollen tubes which were able to penetrate through to the style. Buszard (1983) found flowers from previously defruited trees to have 'firm, expanded' papillae at anthesis whilst those from



Figures 6.4.2.1
Effect of stigmatic surface on pollen tube penetration and growth through the style. The changing condition of the stigmatic surface as time increased beyond 'late balloon' is shown against the number of pollen tubes which initially penetrated it (line A). Also shown for each pollination date is the percentage of pollen tubes which having penetrated the style, subsequently grew through it to points B, C and D. See text for further details.

a) 2-year-old trees
b) 3-year-old trees
c) 5-year-old trees
d) 7-year-old trees

previously heavily cropped trees were collapsed. Williams (1965) although not visually assessing the stigmatic surface, found styles from trees given additional nitrogen the previous autumn to remain receptive for longer than did those from trees not given nitrogen. Together these results may suggest that a good nutritional status of the tree is important in stigmatic receptivity. If so, then perhaps since shoot growth of vertical branches is often more vigorous (Wareing and Nasr 1958), and often continues until later in the season (Kato and Ito 1962) than on horizontal branches, nutritional reserves may not be built up within them and their fruit buds. If this occurs, it may be expressed in depressed stigmatic condition.

As regards 'age of wood' effects, the fact that when flowers from 1- and 2-year-old wood were compared, virtually no differences in stigmatic topography or pollen tube growth were observed, suggests that neither parameter was responsible for the lower setting ability of the flowers on the younger wood.

6.4.3 Ovular condition

Of the two studies of ovular condition during flowering (i.e. 1985 and 1986), the latter is probably more relevant to the orchard situation than is the former. In both years the intention was to examine embryo sacs at increasing lengths of time throughout the flowering period, and to assess whether they would have been capable of achieving fertilisation. In 1985 this was done by examining unpollinated flowers but in 1986 flowers were harvested for examination four days after pollination at increasing lengths of time after 'late balloon'. That pollination itself can increase the length of time that an ovule remains viable has been shown in pear by Herrero and Gascon (1987) who said that 'while in no way altering embryo sac development, pollination induces a prolongation of embryo-sac viability prior to fertilisation taking place'. This is presumably due to the induced stimulated activity within the ovary that has been shown in both cucumber (Fuller and Leopold 1975) and almond (Pimienta and Polito 1983). The presence of auxin in pollen, and its production in the style and ovary accompanying pollen tube growth and fertilisation are well established facts (Nitsch 1952, Crane 1964).

Previous work showing firstly that a wave of cytoplasmic and biochemical activity precedes the pollen tube along the length of the style (Herrero and Dickinson 1979) and secondly that rapid pollen tube growth is associated with conversion of certain gibberellic acids (Kamienska and Pharis 1975) might suggest that a gibberellin or an auxin secreted by the growing pollen tube could be transmitted 'ahead' to the ovule, stimulating it to remain active. That metabolic activity is an important factor in determining the length of ovule viability was shown by Williams (1965). He observed a relationship between cessation of cell division within the ovule and its senescence and resultant abortion and suggested that 'death of the egg apparatus and ovule abortion may be inevitable unless there is continuous cell division in the nucellus up to the time of fertilisation, and afterwards in the embryo sac'.

However, while bearing this in mind, results from the two years of embryo sac examination do not contradict each other. Therefore although in 1985 these embryo sacs were from unpollinated flowers and in 1986 from pollinated ones, useful information regarding ovule condition in relation to fruit set can still be obtained from both investigations.

In both years, flowers from the youngest trees had significantly fewer healthy ovules at 'late balloon' than did those from the older trees. Contributing to this was a higher number of both immature ovules and also of degenerating ones. In 1985 the former was particularly important - 8 days after 'late balloon' 38% of ovules within flowers from the 2-year-old trees remained immature. In 1986, very few ovules remained immature at this time and in flowers from 2-year-old trees, all ovules were degenerate whereas more than half of those in flowers from 5- and 7-year-old trees remained healthy.

Thus in both years ovules within flowers on very young trees appeared to have either arrested, or very slow, development, or else to degenerate very quickly after maturity. Although a genetic predisposition for a high proportion of egg sacs to have retarded development or rapid degeneration has been reported for 'Comice' pear (Jaumien 1968) and 'Delicious' apple (Dorsey 1929, Hartman and Howlet 1954), what is seen here is not inherent within the variety but rather something to do with the particular trees used in this study, most probably their young age.

Embryo sac longevity has long been regarded as one of the major factors determining fruit set and EPP. Roberts (1926) noticed that 'weaker' blossom either had poorly developed, or degenerate embryo sacs - egg cells aborting before fertilisation could take place. And Dorsey, (1929) making the first detailed study of embryo sac development in relation to fruit set, found that in two varieties which usually set badly ('Arkansas' and 'Delicious') embryo sacs degenerated much more rapidly than in varieties ('Jonathon', 'Grimes' and 'Rome') where set was usually good.

It is interesting to see that flowers on branches which had either remained horizontal throughout or else had been tied into a horizontal position in the April prior to flowering were similar to each other in the proportion of healthy ovules they contained, and that those on both vertical branch treatments (although similar to each other) had fewer healthy ovules in comparison. At four or eight days after 'late balloon', flowers from these treatments had a significantly higher proportion of 'healthy' ovules than did those from branches which were either vertical throughout or had been tied up in April prior to flowering.

Flowers from either young trees, vertical branches or 1-year-old wood have a reputation for setting less well than do flowers from older trees (Gardner *et al.* 1952, Forshey 1978), horizontal branches (Greene 1981) or older wood (May 1972). That flowers from these former situations should also display a lower proportion of healthy ovules than did those from the lat-

ter, might suggest that their lower fruit setting ability is due to the same reasons and perhaps, the same causes.

One factor that could affect flowers in both these situations is exposure to the influences of strong vegetative growth. Young trees and upright branches both grow more vigourously than do older trees or horizontal branches (Elfving and Forshey 1976, Forshey 1978). There are two ways in which this might influence ovule condition and ultimately, fruit set. Firstly, if vegetative growth is maintained late into the season, then reproductive buds may be initiated late (Fulford 1966, Luckwill 1970) and therefore enter dormancy in either a less well developed physical or nutritional state (Luckwill 1974). When growth restarts the following spring, these buds will have either more development to do than will buds initiated earlier, or may have fewer nutritional resources to call upon. Both of these could result in production of 'weaker' blossom, as has been shown by Abbott (1970) who manipulated the length of time between flower initiation and dormancy and found flowers allowed only a short period to be of inferior setting quality the following year.

Alternatively, even if reproductive buds on young trees and vertical branches enter dormancy at an equivalent physical and nutritional stage as buds on older trees and horizontal branches, they may still be influenced by differences in shoot growth the following season. Floral buds start development before vegetative ones, but if on young trees/vertical branches, vegetative buds burst and start growing rapidly earlier than they do on older trees/horizontal branches then flowers borne in the former situations will have to compete more strongly for nutrients than will those in the latter. No data regarding the time at which shoot growth starts in different ages of tree is known of but Forshey (1978) reported that vertical shoots started spring growth approximately four days earlier than did horizontal shoots. Abbott (1960) and Quinlan and Preston (1971) both showed that early seasonal shoot growth could be detrimental to fruit set and this would be particularly relevant to the flowers on 1-year-old wood which usually develop several days later than do those on older wood, therefore reaching anthesis when shoot growth has probably already started in earnest.

Unfortunately, although the shoot growth kinetics of various ages of tree and orientations of branch were measured in relation to flower initiation (Chapter 7), no shoot growth measurements were made during the period of flower development/early fruit set.

However, the results obtained here, supported by the fruit set results presented in Chapter 5, show that branch orientation exerts its influence during the period of flower development and growth, rather than during flower initiation the season before. This suggests that it is the early season's growth, rather than growth during the time of flower initiation, which may influence ovary development and fruit set. Because flowers on young trees, vertical branches and young wood suffer from the same type of floral 'weakness', it might suggest that the ad-

vantage conferred by a horizontal branch orientation is an indirect one mediated through decreased shoot growth rather than a direct orientational effect.

6.4.4 Embryo development

In general, the number of embryos developing within flowers from trees of various ages agreed with what might have been expected had the results concerning ovule condition and pollen tube growth been considered.

However, within flowers from 2-year-old trees, no developing embryos were seen; 14 days after pollination all ovules appeared unfertilised and degenerate. This is surprising given that four days after pollination at 'late balloon' 47% of examined ovules seemed healthy (Table 5.3.4.1b); and that 48 hours after pollination an average of 4 pollen tubes had reached the style base (Table 4.3.3.2d). It also contradicts the level of fruit set obtained from pollination at this time, when approximately 22% of pollinated flowers set fruit (Figure 5.4.2.1b).

That this is an anomalous result, probably due to the small sample size (6 flowers) is indicated by the embryo development seen within flowers from the older trees. Here, on average approximately 80% of examined ovules contained developing embryos 14 days after pollination at 'late balloon'. Four days after pollination approximately 45%-60% of ovules within these flowers appeared healthy and approximately 6 pollen tubes had reached the style base 48 hours after pollination. The higher proportion of developing embryos than there had been of healthy ovules suggests that some ovules scored as being 'immature' had continued to develop and were subsequently successfully fertilised.

Of flowers pollinated two days after 'late balloon', the majority of ovules within flowers from 5- and 7-year-old trees had been fertilised, but many had subsequently aborted. Within flowers from the 3-year-old trees 42% had degenerated prior to fertilisation, and 40% after it. It is perhaps interesting that the number of pollen tubes reaching the style base after pollination at this time was extremely low (1.3 tubes). If this is representative of what happened out in the orchard, it may partly explain the poor set. But since the numbers of pollen tubes reaching the style base was higher following pollination two days later, this may be an anomalous result - in which case it suggests that ovules within flowers from 3-year-old trees had a shorter life (as did those from 2-year-old trees) than did those from the older ones.

The presence of so few developing embryos within flowers from any age of tree pollinated four days after 'late balloon' suggest that pollen tubes growing in flowers in the field took more than four days to reach the ovule. Of the flowers on the 5- and 7-year-old trees pollinated four days after 'late balloon' and harvested four days later, more than 50% of ovules appeared healthy, suggesting that if they had been fertilised then, an embryo would have been formed. The fact that no embryos were seen implies that either this visual assessment of egg sac

condition is a poor guide to ovule viability, or else that pollen tubes took so long to reach the style base that ovules had subsequently aborted.

Chapter 7. Effects of tree age, branch orientation and plant growth regulators on shoot growth, floral initiation and development the season prior to flowering.

7.1 Introduction

It has been seen in previous chapters that apple flowers, even when morphologically similar, are variable in their ability to set fruit. Flowers produced in certain orchard situations (i.e. older trees, horizontal branches) set significantly better than those produced in others (younger trees, vertical branches). Differential setting ability of flowers has been shown to occur between trees given different fertiliser applications (Williams 1965), between trees having differing levels of crop the previous year (Buszard 1983), between trees of different vigour (Hansen 1980) and also between trees subjected to different spring temperatures (Miller 1988). Various reasons have been suggested to account for this but little is known about the exact causes. Although some reports show flower 'quality' to be influenced by conditions operating after the previous season's growth has ceased (Hill-Cottingham and Williams 1967, Miller 1988), Abbott (1970) showed very clearly that the length of time between flower initiation and dormancy could also be influential. By inducing flower initiation either early or late in the season he effectively created 'mature' and 'young' buds respectively and found that the flower clusters which developed from buds initiated 'late' were of inferior 'quality' or setting ability compared to those initiated earlier. He suggested that this was due to the late initiated buds having had accelerated development with little time to build reserves.

In apple, as with many other deciduous fruit trees inception of flower buds occurs soon after initial leaf growth during the previous year. Development and growth within the outer bud-scales continues throughout that season (Fulford 1966), perhaps throughout the winter (Hill-Cottingham and Williams 1967), culminating in flowering approximately 10 months after inception.

The exact time of floral initiation varies according to mineral fertilisation regime and growth pattern (Hill-Cottingham and Williams 1967), weather (Abbott 1977), cultivar (Huang 1984), and size of the current crop (Barnard 1938). But although several authors have suggested a close relationship between the timing of shoot growth cessation and floral induction (Barnard and Read 1932, Davis 1957, Abbott in Landsberg 1974) and good correlation between these phenomena is often cited (Hill-Cottingham and Williams 1967, Huang 1984), the stated time interval between the two, varies between authors. Hill-Cottingham and Williams (1967) reported that both occurred almost simultaneously, whereas Barnard and Read (1932), Luckwill and Silva (1969) and Huang (1987) all observed that shoot growth ceased several weeks before floral induction. Similarly, although some people have found the actual time interval between shoot growth cessation and floral initiation to remain constant, in a recent study on pear, Dheim and Browning (1988) found that on trees given certain treatments shoot growth

ceased 2 months earlier than it did on untreated trees but that floral initiation occured 2 weeks earlier on the latter compared to the former. However, comparison of such data is difficult since different authors may use different criteria to identify floral induction.

The reasons suggesting that the timing of shoot growth cessation could be influential in determining the time of flower initiation have already been discussed in Chapter 1 but although many people agree on the general principle, the exact temporal association between the two occurances in uncertain. Whether or not shoot growth has to cease completely (Davis 1957), or simply have a period of reduced growth (Williams 1973) is debatable. Alternatively, some authors report that floral induction may be independent of shoot growth kinetics (Luckwill 1970, Tromp 1972, Dheim and Browning 1987).

However, it was seen in previous chapters firstly that flowers from young trees and vertical branches set fruit less well than do those from older trees and horizontal branches (Chapter 5), and secondly that shoot growth tended to be greater on the former compared to the latter (Chapters 1 and 3). Thus it is feasible that the two things could be connected; the extensive shoot growth found on young trees and vertical branches being detrimental to flower 'quality', perhaps due to the time of flower initiation being delayed.

To investigate this question, shoot growth kinetics and the time of floral initiation were assessed in trees of various ages, branches of different orientations and trees treated with paclobutrazol. The latter treatment was included because paclobutrazol usually decreases shoot growth (Quinlan and Richardson 1984, Stinchcombe *et al.* 1984) and had also been found to be associated with increased fruit set (Chapter 3)

7.2 Materials and methods

This experiment was conducted in 1986, using trees described in section 2.2.1

Five-year-old trees were used to examine the effects of branch orientation, wood age and paclobutrazol application on floral initiation. 2-, 3-, 5- and 7-year-old trees were used to examine the effects of tree age.

Paclobutrazol treatments were allocated using a randomised block design, (blocked on 1986 fruit bud numbers) with eight single tree replicates per treatment. 1000 ppm paclobutrazol was applied to incipient runoff using a hand lance supplied by a hand operated pneumatic sprayer (Cooper Peglar Falcon) on 7th June. Control trees were unsprayed.

On all trees, two branches comprising predominantly horizontal 2-year-old wood and extension shoots were randomly chosen and labelled. On the 5-year-old control trees, two branches with vertical 2-year-old and extension wood were also labelled.

1986 extension growth of the labelled branches was measured weekly; the first measurement being on the 1st July, the last on August 28th.

To assess whether the growth pattern of labelled shoots was representative of that occuring throughout the whole tree, on the 15th July all shoots on the tree were examined and classified as to whether or not they were growing, shoot growth being considered to have stopped when all leaves were unrolled. At subsequent measurement times, all shoot tips on the tree were examined again, and the number still growing counted. Within the trees where both horizontal and vertical branches were being used (5-year-old trees with and without paclobutrazol treatment), seperate counts were made of shoots of each orientation.

The time of floral initiation in each treatment was determined by bud dissection. At each time of shoot measurement, 1-2 non-fruiting spur clusters on 2-year-old wood were collected from each tree (12 clusters per treatment in total), and similar collections were made from about $^1/_3$ of the way up the current year's wood on 5-year-old trees.

Supplementary collections were made at and around the time of shoot growth cessation after which collections continued at approximately fortnightly intervals.

Buds were dissected under a binocular microscope and the number of nodes counted. The shape of the apex was assessed, the condition of the king flower categorised according to the following scheme and the mean score for buds from each situation calculated.

Category	Stage of development
0	Apex flat, vegetative bud.
1	Apex domed, start of floral initiation.
2	Inflorecence initiation
3	Floral initiation, king flower and laterals apparent
4	Sepal initiation
5	Petal initiation
6	Anther initiation
7	Carpel initiation

(adapted from Hill-Cottingham and Williams 1967)

When changes in the apical dome were observed and as its development continued, a few buds from each collection time were quick frozen in Nitrogen slush, coated with gold and observed in a scanning electron microscope.

7.3 Results

7.3.1 Shoot growth kinetics as affected by tree age, branch orientation and paclobutrazol.

On the 1st July mean length of measured shoots varied between 35 cm (on the trees sprayed with paclobutrazol) to 47 cm (on vertical branches) (Figure 7.3.1.1). In horizontal branches on 5- and 7-year-old trees, and trees treated with paclobutrazol, measured shoot growth increased very little after 15th July and although it was seen that approximately 50% of shoots

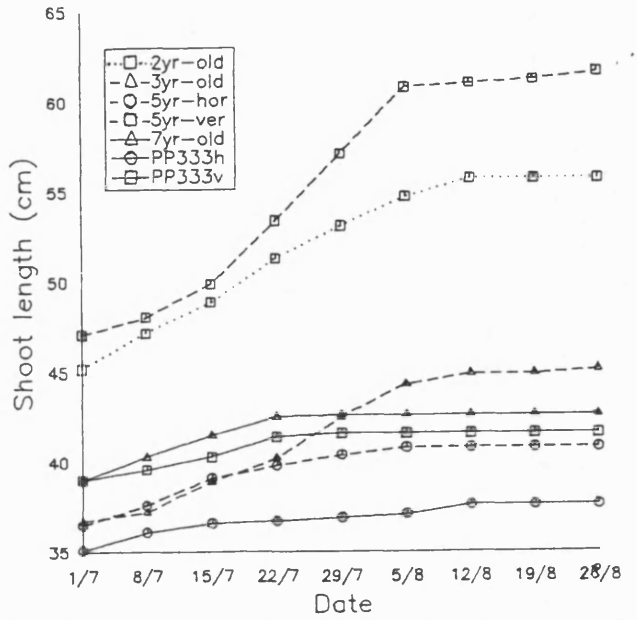


Figure 7.3.1.1.
Length (cm.) of extension shoots on various ages of tree, orientations of branch and trees treated with paclobutrazol (PP333). All branches were horizontal except 5yr-ver and PP333V where they were vertical.

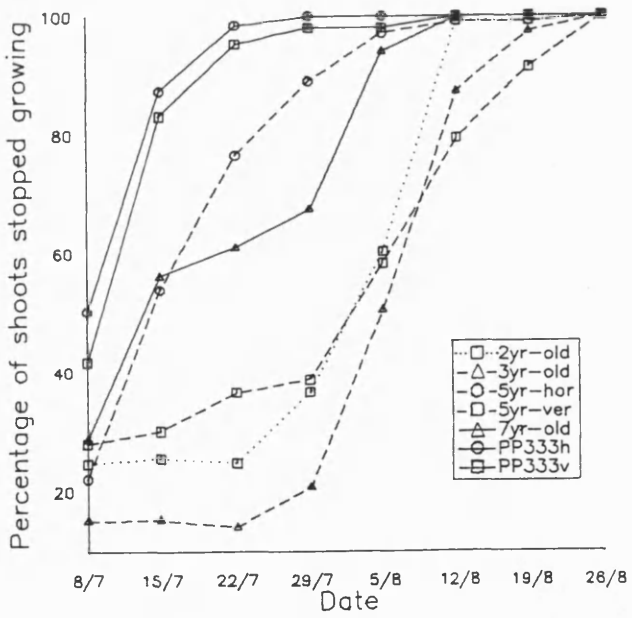


Figure 7.3.1.2.
Percentage of shoots on individual trees, on which all leaves had unrolled indicating extension growth to be stopping. All branches horizontal except 5yr-ver and PP333V where they were vertical.

on the 5- and 7-year-old trees trees were assessed as still growing, 80% of those on trees treated with paclobutrazol had stopped (Figure 7.3.1.2). Within the 2-year-old trees and vertical branches, although some growth continued well into August, vigorous growth of the measured shoots continued until around the 5th and 12th of August respectively by which time shoots in these positions were longer than within any other treatment being 56 cm and 61 cm respectively. Shoots on the 3-year-old trees continued growing until about the same time but their mean length (45 cm) was less than on many other treatments. Calculation of the relative growth rate (cm/metre) showed very clearly that by the end of July, shoots on paclobutrazol treated trees and horizontal branches on 5-year-old trees had completed the majority of their growth whereas those from the other treatments were still growing quite strongly (Figure 7.3.1.3). In particular, shoots on the 2- and 3-year-old trees and vertical branches continued some growth well into August.

7.3.2 Floral initiation and development as affected by age of tree, age of wood, branch orientation and paclobutrazol.

When sampling started in July, excluding those on 1-year-old wood, buds in all positions examined had between 14-15 nodes while axillary buds had 12.6 (Table 7.3.2.1). Node number increased fairly steadily such that by 23rd July all buds on 2-year-old wood had between 18 and 19 nodes. At this time the bud apices were flat, but just over one week later occasional buds within each position showed signs of apical doming, and by the 3rd August the majority of these buds were distinctly domed. Within axillary flowers the first sign of doming was seen on 14th August and by 21st August this extended to the majority of examined buds.

Wihin the first two weeks following the doming of the apex, development within buds from all 2-year-old wood situations proceeded at a similar rate; the apex itself becoming broad and rounded and bract intitials being formed. Between the 14th August and the 27th August the buds increased enormously in complexity; the apex was round and broad with distinct bracts, and lateral flowers and their bracts were easily distinguishable. From an initially very simple structure, the bud suddenly had the initials of several distinct primordia of lateral flowers. During this time (14th-27th August), buds from all 2-year-old wood sampling sites again appeared to have similar rates of development. By 27th August, most axillary buds had reached the stage of a well domed apex, and in occasional buds the apex was flattening out in preparation for king flower differentiation.

During the first two weeks of September development within buds from 2-year-old wood continued rapidly, sepals being formed on the king flower and an increasing number of lateral flowers being initiated. The lateral flowers within each bud ranged in development; when sepals were visible on the most advanced bud, the newest would be small, flat and rectangular with bract initials occasionally identifiable. At any given collection time, stages of development also varied between individual flower buds from the same sampling site. For example,

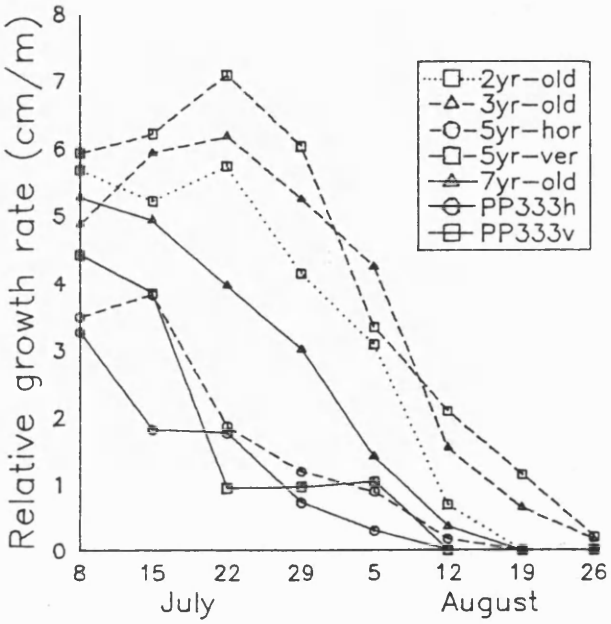


Figure 7.3.1.3
Relative growth rate (cm./m.) of shoots on various ages of tree, orientations of branch and trees treated with paclobutrazol (PP333). All branches were horizontal except 5yr-ver and PP333V where they were vertical.

Table 7.3.2.1 Number of nodes within buds from various ages of tree, orientation of branch on trees treated with Paclobutrazol. Figures in bold represent the time at which apical doming was first observed in each situation examined. All branches were horizontal except where noted below.

Situations used were:-

2yr: 2-year-old trees
3yr: 3-year-old trees
5yr: 5-year-old trees
5yrV: 5-year-old trees, vertical branch
7yr: 7-year-old trees
PP333: 5-year-old trees, treated with Paclobutrazol
PP333V: 5-year-old trees, treated with Paclobutrazol, vertical branches
axillary: current year wood

date	2 yr	3 yr	5 yr	5 yr V	7 yr	PP333	PP333V	axillary
7/7	15·5	15·6	14·1	14·7	15·4	14·7	15·6	12·6
14/7	16·9	17·8	17·1	15·2	17·8	17·1	17·4	14·0
23/7	18·4	18·6	18·7	18·4	18·9	18·4	18·9	15·1
28/7	19·1	19·0	19·2	18·9	19·2	18·9	19·1	16·0
31/7	**19·4**	**19·2**	**19·6**	19·4	**20·1**	**19·6**	**19·6**	16·9
3/8	19·8	19·7	20·2	**19·7**	19·9	20·4	19·7	17·5
7/8	20·0	20·3	21·2	19·9	20·2	20·3	20·2	18·4
14/8	20·1	20·9	21·3	20·3	21·6	20·9	21·1	**19·9**
21/8	21·1	21·3	21·8	20·9	21·4	21·3	21·0	20·6
27/8	21·0	21·9	21·8	21·6	21·7	21·5	21·2	21·3

on the 23rd September, in the least developed bud taken from a 7-year-old tree the king flower had slightly overlapping sepals, was subtended by bracts and had five lateral flowers varying between being rectangular with incipient bracts to being much more rounded with obvious sepals. In contrast, the most developed buds had a king flower quite buried beneath hair-covered bracts under which the sepals were no longer overlapping but were upwardly pointing. These had elongated greatly and were much longer than wide, often with two longer than the others which were pressed flat together. Inside these flowers the primordia of petals and anthers were present. With the king flower in this condition most of the laterals usually had good sepal development.

Although overall, development appeared to be progressing at a similar rate in buds from the 2-, 4- and 6-year-old trees and from horizontal branches of paclobutrazol treated trees, buds from these trees all appeared more advanced than were buds from the other situations. On 5th October buds from vertical branches on paclobutrazol treated trees appeared slightly less advanced than those from the locations listed above but those from the 3-year-old trees, vertical branches on untreated trees and the current years wood were markedly so. At this time, the majority of king flowers within buds on the 2-, 5- and 7-year-old trees had incipient petals and anthers but those from vertical branches had no observable internal differentiation and those from the 3-year-old trees and 1-year-old wood were still at the stage of having unexpanded sepals on the king flower and very little differentiation of the laterals (Figure 7.3.2.1).

Within all buds development continued throughout October, lateral flowers becoming more and more complex such that by the 10th October many buds had several well developed lateral flowers ranging from those with good petal and anther initials to those with only recently initiated sepals. Buds from 3-year-old trees, vertical branches and 1-year-old wood also continued developing but remained slightly less developed than those from other situations such that by 25th October, although some had initiated anthers, these were very rudimentary whereas in the other situations, anther differentiation was underway, petals were expanding and carpel initiation was often visible.

By the last date of sampling (25th October) there were fewer differences in the stage of development reached by the buds, within individual sampling positions although there appeared to be fewer laterals within each bud from 1-year-old wood than there were in those from the other situations.

Fruit set arising from hand pollinations in 1987 was generally very high. Although pollination at anthesis resulted in only 18.2% of axillary flowers producing a fruitlet, the setting of flowers borne in other situations was between 75% and 95% (Table 7.3.2.2). Horizontal branches on the (then) 6- and 8-year-old trees (either with or without paclobutrazol treatment) had the highest level of set (above 90%), whereas those on the vertical branches of the (then) 6-year-

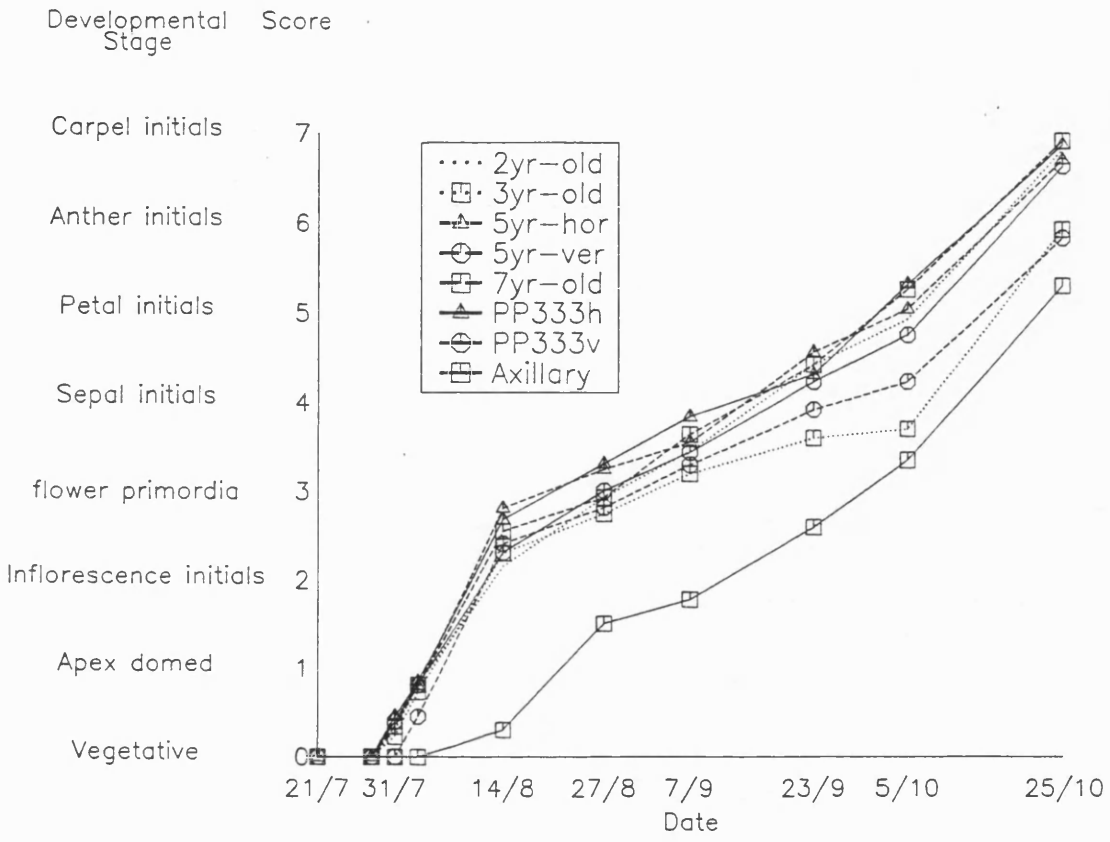


Figure 7.3.2.1
Floral development within buds from various ages of tree, orientations of branch and trees treated with paclobutrazol. At each sampling time buds were scored (0-7), dependant on their stage of development (see text) and the mean score calculated. Mean scores are shown above.

Treatments were:-

2yr-old:	2-year-old trees,
3yr-old:	3-year-old trees,
5yr-hor:	5-year-old trees,
5yr-ver:	5-year-old trees, vertical branches,
7yr-old:	7-year-old trees,
PP333h:	5-year-old trees, horizontal branches sprayed with paclobutrazol,
PP333v:	5-year-old trees, vertical branches sprayed with paclobutrazol,
Axillary:	5-year-old trees, current years wood.

Except where stated otherwise, all branches were horizontal and buds were collected from 2-year-old wood.

Table 7.3.2.2 Leaf and flower number and weight, leaf area, percentage fruit set (and S.E.D.) resulting from hand pollinations at anthesis in 1987 of flowers borne on various ages of tree, orientations of branch and trees treated with Paclobutrazol. Values bearing the same letter are not significantly different at $P \leqslant 0 \cdot 05$.

Situations used were:-

3yr: 3-year-old trees
4yr: 4-year-old trees
6yr: 6-year-old trees
6yrV: 6-year-old trees, vertical branches
8yr: 8-year-old trees
PP333: 6-year-old trees, treated with Paclobutrazol
PP333V: 6-year-old trees, treated with Paclobutrazol, vertical branches
axillary: 1-year-old wood on 6-year-old trees

	3 yr	4 yr	6 yr	6 yr V	8 yr	PP333	PP333V	axillary	(S.E.D.)
leaf area cm.^{2}	44 · 34$_{ab}$	51 · 36$_b$	78 · 89$_e$	67 · 22$_d$	54 · 23$_{bc}$	68 · 34$_{de}$	63 · 14$_{cd}$	34 · 31$_a$	4 · 59
leaf number	6 · 85$_b$	7 · 44$_{bc}$	8 · 63$_d$	7 · 50$_c$	7 · 60$_c$	7 · 41$_{bc}$	7 · 12$_{bc}$	5 · 13$_a$	0 · 277
leaf weight (g.)	1 · 10$_b$	1 · 17$_b$	2 · 03$_e$	1 · 64$_c$	1 · 48$_c$	1 · 82$_d$	1 · 65$_{cd}$	0 · 89$_a$	0 · 08
flower number	6 · 15$_a$	6 · 38$_b$	6 · 06b	6 · 50$_b$	6 · 60$_b$	6 · 34$_b$	6 · 16$_b$	3 · 91$_a$	0 · 233
flower weight (g.)	1 · 086$_b$	1 · 035$_b$	1 · 403$_d$	1 · 342$_c$	1 · 231$_c$	1 · 314$_c$	1 · 257$_c$	0 · 684$_a$	0 · 061
percentage fruit set	85 · 2$_c$	82 · 1$_{bc}$	95 · 6$_d$	75 · 9$_b$	92 · 9$_{cd}$	91 · 6$_{cd}$	86 · 4$_c$	18 · 2$_a$	3 · 9

old trees set least (75%). Intermediate were flowers on the younger trees and on vertical branches of the paclobutrazol treated trees.

Clusters produced the following year showed that axillary buds had reduced flower numbers (3.9) compared to buds from 2-year- old wood (6.1- 6.7). However, although flower numbers may have been similar in the buds produced on 2-year-old wood, flower weight was not. It was highest in the flowers from horizontal branches on 6-year-old trees and lowest in flowers from 3- and 4-year-old trees. When flower weight was divided by flower number to obtain a rough estimate of individual flower weight, flowers on 3- and 4-year-old trees and those from axillary buds were significantly lighter than those from all other situations.

Similarly, cluster leaves from horizontal branches of 6-year-old trees were more numerous, heavier and with a greater leaf area than was found in clusters from all other situations. Those from axillary buds had fewer, lighter leaves and with a lesser leaf area than did clusters from all other situations which were intermediate in all these characters.

7.4 Discussion.

Several points regarding the growth and flower production of young trees, different branch orientations and ages of wood arose from this experiment. Firstly, as is often reported (Luckwill 1974, Forshey 1978), the younger trees were more vigorous than the older ones and generally continued growing later in the season. This was most pronounced on the 2- and 3-year-old trees. Also, as is similarly reported (Kato and Ito 1962), vertical shoots grew more strongly, and until later in the season than did horizontal shoots on the same tree.

It had been suggested that differences in shoot growth kinetics within the various orchard situations examined may affect the time at which floral initiation occurred but this did not appear to be the case. In all buds from 2-year-old wood, the number of nodes present at the start of sampling, and rate of node production during July was very similar. Once approximately 20 nodes had been formed, apical doming (taken as the first sign of flower induction) occurred with remarkable synchrony throughout all examined situations. This was first seen in buds from vertical branches on August 3rd, and had been observed at the previous sampling time three days earlier in buds from all other 2-year-old wood situations. This synchrony of flower induction has also been reported by Luckwill and Silva (1979). However, Luckwill and Silva were assessing trees of one particular age, in one orchard, upon which various treatments were imposed. It is perhaps more remarkable that a similar result has been found here, where there were a range of tree maturities and orchard locations.

However, compared to the buds from 2-year-old wood, those from axillary positions were approximately two weeks behind in their development, reaching the stage of apical doming on 14th August. This is in agreement with Zeller (1960) and Dheim and Browning (1988) who found flower initiation in axillary buds (of apple and pear respectively) to be delayed com-

pared to that in spur buds. However, in this study, during the month following apical doming inflorescence initiation and flower initiation occurred at much the same rate in buds from all situations.

What is interesting about flower initiation occurring so synchronously on all the 2-year-old wood throughout the range of situations examined is that this did not appear to be entirely linked with the time of shoot growth cessation. At the time apical doming was first seen, both horizontal and vertical shoots of the paclobutrazol treated trees and the horizontal shoots on the 5-year-old trees had produced virtually no growth during the previous three weeks. Thus in these situations there was a slight delay between shoot growth cessation and floral initiation. In contrast, at this time of apical doming, shoots on 2- and 3-year-old trees were still increasing in length, apparently without any inhibitory effect on floral initiation. An intermediate pattern occurred on the vertical branches of the 5-year-old trees where apical doming and the cessation of measured shoot growth appeared to coincide (both being observed to occur on 3rd August). These results are in agreement with those of Dheim and Browning (1988) who found that flower initiation in spurs of Doyenne du Comice pear trees occured on the same date (30th July) both on control trees and on those sprayed with 1000 ppm paclobutrazol even though shoot growth stopped on 25th July on the latter and on 20th August on the former. Others however, report differently. Hill-Cottingham and Williams (1967) found that summer nitrogen applications to Golden Delicious trees resulted in an extended period of shoot growth and a correspondingly delayed time of floral initiation; and Banno *et al.* (1985) found that bending shoots of Japanese pear to a horizontal position resulted in their growth stopping 20 days earlier than equivalent upright shoots and the timing of flower initiation in axillary buds to be advanced by 1 month in the former.

Because examined buds were collected from the relevant situations throughout entire trees and not just from the wood subtending the measured branches, it could of course be argued that the shoots selected for measurement were not representative of the general situation. However, assessment of the proportion of shoots still growing on each tree was in good agreement with individual shoot measurement, shoots on younger trees and vertical branches (of non paclobutrazol treated trees) continuing growth later in the season than did those from older trees and horizontal branches.

Thus although many people have suggested that a strong correlation exists between the time of shoot growth cessation and flower initiation (Davis 1957, Hill-Cottingham and Williams 1967) others have found little temporal connection between the two phenomena (Barnard and Read 1938, Dheim and Browning 1988) and from this experiment it would appear that any such association which may exist is not of a constant degree, perhaps because there may be some other factor(s) with overriding control. However, flower initiation did occur 'around'

the time of shoot growth cessation so the possibility cannot be ruled out that this may play a crucial role in determining the time of floral induction.

Once induction had occurred, development within buds taken from all examined situations proceeded synchronously for about four weeks before becoming divergent. Although axillary buds continued to have a parallel, if delayed course of development compared to buds on 2-, 5- and 7-year-old trees and those treated with paclobutrazol, buds on vertical branches and 3-year-old trees had a slightly slower rate of development than did those from other situations. Why there should be this divergence of developmental rate is unclear. However it has been shown that nutritional factors as well as hormonal ones are important in bud development - Hill-Cottingham and Williams (1967) showing that late nitrogen application after shoot growth had stopped could accelerate bud development, - therefore it might be suggested that in the 3-year-old trees and vertical branches some nutrient was limiting. Presumably since the vertical branches were on the same trees as the horizontal branches this could not be a nutrition deficiency inherent in the orchard but is is possible that early leaf loss from the vertical branches may have been contributory. If this had occurred it would have reduced both the carbohydrate supply to the buds and also the supply of other nutrients delivered to them through the transpiration stream. Unfortunately no records of leaf loss were taken. Another point of interest here is that it was the buds on the 3-year-old trees rather than those on the 2-year-olds which had the slower developmental rate whereas shoot growth was greater on the latter compared to the former. However, in terms of relative growth (cm/m) the 3-year-olds were as active during August as were the vertical branches of 5-year-old trees and perhaps slightly more so than were the 2-year-old trees (Figure 7.3.1.3). Although all growth had ceased during the time of divergent developmental rates, growth which continues late into the season may, depress the levels to which nutrients are stored within the tree. Experiments where growth regulators have induced an early cessation of shoot growth have shown that although extension growth has stopped, shoot diameter continues to increase, and at a greater rate than in treatments where shoot growth continued (Wieland and Wample 1985). This presumably reflects a higher level of nutrient storage in the former.

Having seen that axillary buds became floral at a later time than did spur buds, and that axillary buds, plus buds from 3-year-old trees and vertical branches were at a less developed stage at the last sampling time, it might suggest that the flower clusters formed in these situations would be 'weaker' than those borne in the other circumstances examined. This was found to be partly true.

Firstly, the clusters produced from axillary buds had a reduced flower number and flower weight, smaller leaf area and leaf weight, and a very reduced fruit setting capacity. This cluster morphology is partly in agreement with that of the 'young' clusters observed by Abbott (1970). He found flower number and fruit setting ability to be reduced by late flower initiation,

but, he also found these clusters to have large primary leaves and not the reduced area found here.

Secondly, clusters from vertical branches also had reduced setting ability compared to those from horizontal branches on the same tree and on paclobutrazol treated trees, but this was not associated with any great reduction in leaf or flower number or weight. Although leaf area and leaf weight were significantly less on the vertical branches compared to the horizontal ones, they were similar to those found on the paclobutrazol treated trees where fruit set was significantly higher.

Of interest here is that spraying trees with paclobutrazol in July had somehow negated the deleterious effects that a vertical branch orientation has on flower 'quality' and fruit set. When comparing clusters from the two vertical branch situations, it was seen that although fruit set had been increased from 75% to 84% by paclobutrazol application, the only detectable difference between clusters was that those from paclobutrazol treated trees had slightly heavier leaves. Whether this was contributory to the improved setting ability of these clusters in unknown.

Thirdly, although buds from 2-year-old trees had developed at the same rate as those on older trees, whilst those on 3-year-old trees developed more slowly, the fruit set of clusters from the two youngest tree ages were very similar (85% and 82% respectively) and the clusters on 2-year-old trees appeared to have slighter slightly lower leaf area and leaf weight. This implies that the advanced state of development achieved by buds on the 2-year-old trees compared to the 3-year-olds by the end of October had not conferred an enhanced setting ability. Also, although bud development in the youngest trees appeared synchronous with that on the older trees, clusters were smaller and set fewer fruit. Unfortunately no measure of bud weight or size was made, so it is not possible to determine whether or not these buds were in fact smaller and less 'strong' even prior to dormancy than were those from the older trees. An alternative explanation for the reduction in cluster size and setting ability despite similar bud development is that conditions during the winter or during spring development and flowering may be particularly detrimental to flowers on young trees. Perhaps the most likely reason is that the vegetative growth may start earlier and more vigorously within these situations and thus flowers will be under strong competition for metabolites.

Chapter 8 . Summary and conclusions.

8.1 Summary of work conducted.

Within this project it was intended to investigate the reasons underlying poor productivity in young orchards (particularly Cox) and to explore the potential for improving it. To this end a broad range of investigations have been conducted.

Firstly, a study was made of the manner in which the individual components of cropping and growth (flower bud number, initial fruit set, final fruit set and shoot growth) varied according to tree age. This was conducted in two successive years. In 1985 2-, 3-, 4-, 6- and 12-year-old trees were assessed, in 1986 2-, 3-, 5- and 7-year-old trees were used (Chapter 2).

Concurrent with this study, growth regulator and cultural techniques were applied to young (2-year-old) Cox and Bramley trees in order to evaluate their potential for improving the cropping of such trees. Paclobutrazol, daminozide, gibberellin, shoot-tipping and training branches to a horizontal position were all investigated as to their effects both on vegetative growth and also on cropping and its components (Chapter 3).

Having established that lack of initial set, rather than sparse flower production may be the main contributory cause of the poor cropping of young trees (Chapter 2), a study was made of developing flower clusters (from bud burst to anthesis) borne on trees of increasing age. This was intended to assess whether there were any differences in cluster morphology, mineral content, chlorophyll content or carbon dioxide uptake that might be adversely affecting the fruit setting ability of flowers on young trees (Chapter 4).

Following on from the study of flower cluster development and morphology, a more detailed examination of the fruit setting ability of flowers borne in several situations was made (Chapter 5). Within this, the female fertility and length of time during which pollination resulted in successful fruit set (the effective pollination period) was assessed in;

(a) different ages of wood and orientations of branch within a tree,

(b) various ages of tree (2- to 12-year-old in 1985, 2- to 7-year-old in 1986),

(c) branches subjected to various shoot tipping or branch orientation treatments.

Results having shown that both female fertility and length of effective pollination period were lower in flowers borne on young trees (2- and 3-year-olds) and vertical branches than they were in older trees (more than 4-years-old) and horizontal branches (Chapter 5) a more detailed study of the factors contributing to this was made.

This involved an assessment of the components of fruit set itself (stigma receptivity, pollen tube growth, ovular condition and embryo development) within flowers from all the above situations (Chapter 6).

Finally, to complete the circle of investigation, having found that flowers borne on young trees or vertical branches had lower fruit setting abilities than did those on older trees and

horizontal branches, and that flowers on paclobutrazol or daminozide treated trees set fruit more readily than did those on untreated trees, a study was made to determine whether differences in shoot growth the preceding season had exerted any influence on either the timing of flower initiation or the rate of flower bud development, prior to dormancy (Chapter 7).

8.2 Effects of tree and wood age on fruit production.

From these studies it was found that the young trees did produce flowers, and expressed on the basis of tree crown volume, these trees produced as many flowers as did older, cropping trees (Chapter 2). It was also found that very few of these flowers were initially set, and although even fewer were retained until final set, lack of initial set appeared the major contributor to the poor fruit productivity.

Closer examination of the fruit setting abilities of flowers on the various ages of tree (by hand pollinations progressively delayed beyond the 'late balloon' stage) showed that at 'late balloon' and beyond, flowers on young trees had a much lower capacity for fruit set than did those on older trees, and also that the time period during which pollination could result in fruit set (the EPP) was much shorter on the young trees (Chapter 5). Thus the chance of flowers on these young trees producing a fruit under natural orchard conditions were much reduced. Even if pollination occurred immediately the flowers opened, fruit set would be low, and if pollination should be delayed for even a couple of days (perhaps due to adverse weather conditions) then fruit set might not occur at all.

Detailed examination of how the individual components of fruit set (i.e. stigmatic receptivity, pollen tube growth, ovule longevity and embryo development) varied with tree age identified the main problem areas (Chapter 6).

In 1985 it appeared that stigmatic 'health' (i.e. the proportion of papillae which are turgid and free from excess secretions) might be a factor contributing to the poor fruit set in young trees - at all times of examination from 'late balloon' to 10 days later, stigmas from 2-year-old-trees consistently had a lower proportion of healthy papillae than did those from older trees. However, this pattern was not repeated in 1986 - flowers from all trees having a similar proportion of healthy papillae on each date of sampling. Although time associated changes in stigmatic topography have been observed previously in both apple (Braun and Stosser 1985, Miller 1988) and pear (Herrero 1983), how such changes in the stigmatic surface actually affect fruit set had been little investigated. From the 1985 data in particular there would appear to be little correlation between the assessed stigmatic condition and the level of fruit set obtained. Although stigmatic condition deteriorated steadily as time beyond 'late balloon' increased (such that 4 days after 'late balloon' only 60 % of papillae appeared 'healthy' within the flowers from 3- to 12-year-old trees) levels of fruit set obtained on the same trees re-

mained high throughout this period (Chapter 5). Indeed, following pollination 4 days after 'late balloon' fruit set on all ages of tree was slightly, but not significantly higher, than had been obtained from pollinations earlier than this.

Similarly, although stigmas within flowers from the 3- to 12-year-old trees had almost identical proportions of healthy papillae at each time of sampling, the fruit set obtained from hand pollinations on the same days was consistently and significantly higher on the 6- and 12-year-old trees compared to the 3- and 4-year-old trees. Herrero (1983) reported that in pear "the stigmatic surface that supports pollen germination offers the typical disorganised appearance of receptive stigmas". However, although slightly higher levels of fruit set were obtained from pollinations conducted 4 days after 'late balloon' at a time when stigmatic 'disorganisation' had begun, these were not significantly different from those arising from pollinations conducted at 'late balloon' itself when the majority of papillae were turgid. Thus results here do not provide any support for this statement being applied to apple. In contrast to the results of Braun and Stosser (1985), the number of pollen tubes penetrating the style decreased as time after 'late balloon' increased, presumably due to the changes in stigmatic condition. Although some pollen tubes grew through the styles of flowers of the young trees following pollinations 8 or 10 days after 'late balloon' very few reached the style base. The fact that some tubes did grow the whole length of the style following pollination at this time shows that stigmatic and stylar condition was not completely inhibiting pollen germination and tube growth even though these were both markedly reduced. It also suggests that in the circumstances here pollen tube growth was not the cause of poor fruit set found in these trees. It remains possible though that given certain orchard or climatic conditions (a desiccating wind perhaps) stigmatic and stylar degradation could be a major cause of poor poor fruit set.

Within these results, apart from the reduced stigmatic condition of the youngest trees in 1985, no consistent tree age effects were seen, either in the condition of the stigmatic surface or in the number of pollen tubes growing through the style. Thus if stigmatic condition and pollen tube growth were generally unaffected by the age of tree upon which flowers were borne, although fruit set was, then ovular condition or embryo development must have been responsible for the poor fruit set seen in young trees.

Ovular condition was indeed seen to vary between the trees of differing ages. Both Fulford (1965) and Williams (1970) reported that (in diploid cultivars) the final stages of egg sac development occurred close to anthesis. Williams (1970) also reported that triploid cultivars have an extended EPP compared to diploid ones and suggested that this might be partly due to the delayed time of egg sac maturation found in the former. However, in 1985, only 17% of ovules in flowers from 2-year-old trees had mature egg sacs, 30% already being degenerate and 52% being immature. Unlike the situation in triploid varieties, the majority of egg-sacs which were immature at anthesis did not continue development to maturity, but rather re-

mained in an immature state, 47% still being assessed as such in flowers harvested 8 days after 'late balloon'.

In 1986, an investigation of how components of fruit set varied with tree age was conducted in a manner perhaps more relevant to the natural field situation - i.e. flowers were pollinated at increasing lengths of time after 'late balloon' and the condition of ovules and embryos assessed following harvest 4 or 14 days later respectively. Although within flowers from the older trees the majority of ovules were mature and healthy 4 days after pollination at 'late balloon', within the youngest trees less than 50% of ovules were in this condition, nearly 40% being already degenerate. Because, according to the Pollen Tube Index of Williams (1970b), pollen tubes would have required at least 4 days to grow from the stigma to the ovaries, a large proportion of egg-sacs would already have been beyond the capability of being fertilised by the time the pollen tube reached them, even if natural pollination had occurred immediately the flowers opened.

Following pollination 4 days after 'late balloon' no mature and healthy ovules were visible 4 days later in the flowers from the 2-year-old trees, and only 22% of those in flowers from the 3-year-old trees were classified as healthy at this time. This suggests that little, (in the 3-year-old trees) or no (in the youngest trees) fertilisation and fruit set was possible following pollination at this time. This was in good agreement with the levels fruit set obtained from pollinations throughout flowering (Chapter 5). Fertilisation, although necessary for fruit set is not the sole determinant, and continued development of the embryo is needed if seeds are to be formed and fruit to be set (Luckwill 1953). In general the number of developing embryos within fruitlets collected 14 days after pollination was in good agreement with what might have been expected from a consideration of ovular condition and pollen tube growth data. It showed that following pollination at 'late balloon' many ovules within flowers from the older trees had been fertilised and were sucessfully developing embryos, and also that the number of these decreased when pollination was delayed by four days. However, although approximately 18% of flowers on 2-year-old trees successfully set fruit following their pollination at 'late balloon', no developing embryos were seen within flowers collected from them 14 days after these pollinations. Because of the large difference in sample size (100 flowers for fruit set determination and only 6 for embryo sac assessment) this discrepency is probably an anomaly caused by the small samples used for assessing embryo development rather than indicating that no seed were formed.

Thus it would appear that the lack of fruit set found on these young trees was caused by a combination of either a premature cessation of egg-sac development and/or accelerated egg-sac development/degeneration, such that even under optimum pollination conditions, fertilisation was unlikely to occur. Problems with egg-sac development/early degeneration have previously been reported as a cause of poor fruit set - from Hartman and Howlett (1954) cit-

ing either a tardy megaspore development or rapid breakdown of egg-sacs as a reason for the poor setting of 'Delicious' trees, to Miller (1988) finding that the main cause of the reduced fruit set associated with warm spring temperatures was that they advanced embryo-sac development to such an extent that the majority of these were fully mature at anthesis but had degenerated prior to the arrival of pollen tubes several days later. This early degeneration of egg-sacs would also appear to be the ultimate cause of poor fruit set within axillary flowers, 79% of their egg sacs being degenerate 4 days after pollination 4 days after 'late balloon'. Why this should occur is uncertain but may somehow reflect a lack of post-flowering support. This might perhaps be due to an inadequate leaf surface for photosynthate provision, or inadequate vascular connections to the main tree (neccesary to obtain metabolites from the roots). Alternatively, because axillary flowers reach anthesis later than do those on older wood, and at a time when shoot tips will have started growth, they are likely to be subjected to stronger competition for communally available nutrients.

Examination of flower cluster development from bud 'burst' to 'anthesis' on the various ages of tree showed that in both 1985 and 1987 clusters from the youngest trees had a lower leaf area and weight, and lower flower number and weight than did clusters from older trees (Chapters 4 and 7). Although leaf area is of crucial importance to cluster development and fruit set (Hansen 1980, Ferree and Palmer 1982) being both the source of photosynthates for flower and fruitlet growth and development, and also by maintaining the flow of nutrients up from the roots via the transpiration stream, small leaves are not always associated with poor fruit set. Abbott (1970, 1984) found that 'young' clusters with large leaves set fruit less well than did 'old' clusters with small leaves, and found that fruit set was not influenced by a reduction in leaf area (from 80-100 cm^2 on 'mature' clusters to around 15-20 cm^2 on 'old' ones,) of far greater magnitude than observed here (in 1985, from around 65cm^2 on 4-year-old trees to c 25cm^2 on 2-year-old trees). However, although Abbott concluded that reduced leaf area did not affect fruit set in his experiment, these differences in area had been induced by (presumably) very different causes to the ones operating here. The reductions in leaf area in his experiments were induced by lengthening the time interval between floral initiation and dormancy, whereas here, (Chapter 7) it was shown that floral initiation occurred almost simultaneously within all ages of tree examined and all were thereafter subjected to the same winter conditions. However, it was also shown (Chapter 7) that shoot growth continued later into the season on young trees compared to the older ones and this may have contributed to the production of small flower clusters. It is widely accepted that the interval between cessation of extension shoot growth and abscission of leaves is an important time for the storage of carbohydrates within the tree (Priestley 1964, Ferree 1981). Carbohydrates produced within the leaves are, in the absence of a fruit crop, available for storage either in developing flowers or within the woody tissues and roots. Thus if for any reason shoot growth continues late into the

season and/or leaf abscission occurs early, then less carbohydrate may be available for both floral development and nutrient storage. Extended shoot growth could therefore conceivably affect the nutrition available for developing buds and perhaps the developmental and nutritional state with which they enter dormancy. Unfortunately bud weight throughout development was not recorded, and although floral development appeared in one case to be uninfluenced by shoot growth (2-year-old trees), in another, extended shoot growth and delayed floral development occurred within the same trees (3-year-olds). However, flower clusters the following year were very morphologically similar between these two situations, both being of lesser 'quality' than were clusters from situations where growth had stopped earlier the previous year. However, in addition to floral development prior to dormancy affecting the clusters produced, the amount of carbohydrate stored within the tree itself may affect flower 'quality' and fruit set. Because initial bud development in the spring relies upon stored resources (Priestley 1960), if extended shoot growth had detracted from the build up of these resources, then bud and cluster development may have been adversely affected. Although ultimately cluster leaves will supply the majority of carbohydrates for flower/fruitlet development, leaves are initially importers of carbohydrate, only starting to export once they reach a certain size. This change from 'importer' to 'exporter' often occurs around the time of 'pink bud' (Quinlan pers. comm.), which is also the time that floral abortion and abscission occurred in the clusters on the youngest trees (Chapter 4). If at this time flowers usually become dependent on a supply of leaf photosynthate for their continued growth, the occurrence of floral abscission at this time might suggest that, in the young trees, this supply was inadequate.

The fact that no differences in chlorophyll or mineral concentration were detected between the clusters from the various tree ages suggests that deficiency in these was not a major cause of depressed set, and that leaf size itself may have been more important.

In agreement with this was the cluster development of axillary buds (Chapter 7). Floral initiation was found to occur approximately two weeks later in axillary buds on current years wood than in those on older wood, and although they then developed at a similar rate to buds from 2-year-old wood, ultimately they were at a less developed stage by the end of October. Clusters developed from these buds the following year were smaller in leaf area, had less leaf and flower weight and fewer flowers (presumably due to lack of initiation rather than floral abortion in the spring) than had clusters on 2-year-old wood. Similar to clusters on the various ages of tree, there were no detected differences in chlorophyll or mineral concentration between axillary flowers compared to clusters from 2-year-old wood on older trees, and thus nothing to account for their poor set in comparison.

Although it has been shown that reduced leaf area is not necessarily detrimental to fruit set (Abbott 1970), this does not rule out the possibility that reduced flower size/weight might be. Indeed good 'quality' flowers are often reported to be large or bold compared to other, less

good 'quality' ones (Hill-Cottingham and Williams 1967, Goldwin 1978, Abbott 1984). In both situations where floral fruit setting abilities were markedly reduced (young trees and 1-year-old wood), flower weight and size (measured as receptacle diameter and pedicel length) were also decreased compared to flowers from situations where fruit set was better (older trees, older wood) suggesting that those from the former situations may have been inherently weaker than those from the latter. Again this might be a consequence of them having been initiated late the previous year and having had a greater proportion of their development to do prior to flowering in the spring. This hypothesis was substantiated both by bud dissections - buds from young trees and 1-year-old wood were at a less well developed state in October compared to those from other situations (Chapter 7) - and also the late development of clusters from these situations in spring (Chapter 2). Late development in spring has previously been associated with poor fruit set (Roberts 1947, Buszard 1983) and was thought to be due to an accelerated rate of development occurring within the flowers. Another factor which may be involved is that if clusters develop late in one part of the tree compared to another (as with axillary flowers) then at the time of their flowering and potential fruit set, other flowers will already be in the stage of early fruitlet development and will be acting as strong metabolic sinks thus discouraging nutrients from being directed to the delayed flowers and thereby further disadvantaging them. Also, even in situations where flowering is synchronous throughout the whole tree, because shoots and flowers/fruitlets are in strong competition, if flowering is delayed shoot tips may already have started growth by the time anthesis is reached, and their increased 'sink' strength will tend to reduce nutrient supply to the flowers. This might perhaps prevent the flowers from becoming 'strong' and setting well. Obviously late flowering is a physiological disadvantage which will serve to make fruit set more difficult.

Within the studies of fruit set potential (Chapter 5) it was found that flowers on 2-year-old wood consistently set better than did those on 3-year-old wood within the same trees. However no major differences in cluster development, leaf or flower size, mineral or chlorophyll concentrations were found. Possible causes of this depressed fruit set are firstly, that by being almost invariably, further into the crown of the tree, clusters on 3-year-old wood may be adversely affected by shading - the effects of which are well known to be deleterious to fruit set (Jackson and Palmer 1977). Secondly, it may be caused by the presence of fruits on the same wood the previous year . 3-year-old wood is more likely to have already borne fruit than is 2-year-old wood, and the presence of fruits has been shown to be both inhibitory to flower initiation (Chan and Cain 1967) and depressive of flower 'quality' the following year (Williams *et al.* 1980).

8.3 Effect of branch angle on fruit production.

In trying to elucidate where the possible reasons for poor ovular condition and its expression in poor fruit set might lie, it is interesting to see the manner in which branch orientation affected this. Horizontal branches are reported to encourage fruitfulness (Preston 1974, Forshey 1978, Greene 1981) and in the experiments described here, they consistently bore flowers with a greater setting ability than did vertical branches. These flowers had both a higher set potential when pollinated at 'late balloon' and also a longer EPP (Chapter 5) - a combination with the potential to greatly increase fruit set. When the underlying causes of this were examined it was seen that the main cause of the increased fruit set of flowers on horizontal branches appeared to be due to their greater proportion of healthy ovules, both at anthesis and beyond, than flowers from vertical branches (Chapter 6). Unlike clusters from young trees or 1-year-old wood, there were no large differences in cluster morphology between flowers which set well (on horizontal branches) and those which set badly (on vertical branches). Although it was seen that development within buds on vertical branches might reach a less advanced state prior to dormancy than would buds on horizontal branches (Chapter 7) there were few differences in cluster morphology the following year. Therefore in both years of assessment, clusters from both branch orientations had very similar leaf and flower sizes and weights, mineral and chlorophyll concentrations, and leaf area.

Of great interest was the manner in which treatments imposed on branches of differing orientations could alter the 'quality' of flowers produced upon them. Although floral development during the autumn prior to flowering was seen to be influenced by branch orientation (Chapter 7), fruit set was more affected by the orientation of branches during the flowering period itself (Chapter 5). Indeed, altering branch angle from upright to horizontal at as late a stage as 'pink bud' resulted in fruit set levels equivalent to those on branches which had been horizontal throughout. Similarly, the fruit set of flowers on horizontal branches which had been tied into a vertical position at 'pink bud' was significantly less than that occurring on branches which had remained horizontal, and was very similar to that achieved on branches which had been vertical throughout.

These differences in fruit set levels were also reflected in assessments of ovular condition (Chapter 6). Flowers from horizontal branches had a greater proportion of their ovules in good condition 4 days after pollination at 'late balloon' than did those from vertical branches, and changing branch orientation at 'pink bud' altered this greatly. Flowers from the branches which were changed from a horizontal position to a vertical one at the 'pink bud' stage had a lower (c 56%) proportion of healthy ovules than did those from branches which remained horizontal throughout flowering (c 70%). Thus in this case orientation must have been exerting its effect at a time very close to anthesis and/or during initial fruit set.

When branches are trained to a horizontal orientation, the apical dominance and corresponding activity within the shoot tip itself is reduced (Wareing and Nasr 1961) with a resultant decrease in hormone activity (Kato and Ito 1962); hormones and nutrients may also be redistributed between the branches (Smith and Wareing 1965). Thus improved fruit setting of flowers on horizontal branches may be associated either directly with these phenomena, or perhaps more indirectly through the associated modification of shoot growth. If vertical branches start growing before, or more strongly, than do horizontal branches (as shown by Forshey 1978) they may become strong competitors for nutrients working against the flowers and/or fruitlets. This hypothesis would also tie in with the theory that early shoot growth of young trees may be inhibitory to fruit set occurring within their flowers.

It might be expected that if early shoot growth of vertical branches is the reason for the poor fruit set found on these branches then removal of shoot tips during flowering might negate this. However, removal of shoot tips from both vertical and horizontal branches either in autumn during floral initiation and development, or when clusters were at the 'pink bud' stage did not significantly affect fruit set at all (Chapter 5). Under these treatments flowers on horizontal branches again consistently set better than did flowers on vertical branches independently of whether shoot tips had been removed or not. Thus either shoot tip activity is not an important factor affecting flower development and fruit set, or else although shoot tips have been removed, new buds quickly grew out in their place and nullified the treatment.

So, overall, in the three situations where cropping is generally expected to be poor (i.e. young trees, axillary flowers and vertical branches) it was seen that flowers produced within them had a lower proportion of viable ovules (due to either inhibited development or more commonly, early degeneration) at a time when fertilisation might have occurred (i.e. about four days beyond anthesis) than did flowers from the complementary situations where fruit set is usually better - i.e. older trees, older wood, horizontal branches.

In the first two situations this occurred within clusters which were physically smaller than were those which had a higher proportion of healthy ovules (older trees, older wood), but in the 3rd (vertical branches), no differences in cluster morphology were seen between these and their better setting comparisons on horizontal branches. Although the ultimate cause of poor set may be the same in the three situations, the factors inducing it may well differ. However, it would appear that all these situations associated with poor set are ones in which vegetative growth might be expected to be strong - the vigorous growth associated with young trees and vertical branches might have the same ultimate effect as delayed cluster development has on axillary flowers - i.e. flowering and fruit set may well be occurring in the presence of competition from shoot growth that is already strongly underway. Compounding these effects might be a lack of nutrient storage and/or bud development the previous autumn. This might appear to be of relevance to the different ages of tree where resultant clusters were

generally smaller on young trees compared to the older ones (Chapters 4 and 7) and where defoliated clusters could still set fruit on older trees but not on younger ones (Chapter 5) but did not seem so important on the branches of differing orientations where clusters were morphologically very similar.

In summary, it would appear that in order initially to overcome the propensity of young trees for strong vegetative growth and minimal cropping, two orchard management practises might be beneficial. Firstly, because branch orientation influenced the fruit setting abilities of flowers during the flowering period itself rather than flower formation, and because the influence of the shoot tip itself was so crucial to this, it would seem important that during winter management, shoot tips of as many branches as possible were made horizontal. This alone may be sufficient to induce some flowers to set fruit and thus start the tree on the road to having a better balance between growth and fruit production.

Secondly, it would seem that further work evaluating the effects of shoot tipping, continuously throughout flowering if necessary, (even if only for one year) might reduce shoot competition sufficiently at a critical time to allow flowers to obtain sufficient nutritional resources to remain viable for longer and thus perhaps, set fruit.

8.4 Potential for increasing productivity of young, extensively planted orchards.

Of the investigated methods for improving productivity of young trees in an extensively planted orchard, only tying down branches to a horizontal position can be wholeheartedly recommended (Chapter 3). This technique improved both flower production and fruit set, and although there was some shoot growth reduction, this was not excessive. The other techniques were either largely ineffective (gibberellin application and shoot tipping) or severely reduced shoot growth albeit with an increase in flower production and fruit set (daminozide or paclobutrazol application). Although paclobutrazol appeared successful at increasing fruit production on Cox trees on either rootstock and there was no associated growth reduction, the same treatment applied to Bramley resulted in a severe reduction in shoot growth. Thus, although in this case the treatment was successful in Cox, extensive testing would be necessary before being confident that it could be used in a commercial situation, where any severe reduction in growth (as occurred in the Bramley) would be undesirable.

It is highly unlikely that altering shoot orientation would ever inhibit growth so severely that major damage was done in steadily filling the orchard with bearing wood. But, should this be thought to be occurring, allowing shoot tips to grow in an upright direction rather than being restrained horizontally would alleviate this affect almost immediately. In contrast, if severe restriction of shoot growth and orchard filling occurred following a chemical application (as happened in these experiments), the effects may persist within the trees for several years (Stembridge and Ferree 1969, Southwick *et al.* 1973) and very little could be done to over-

come them. By this manner, severe damage could be done to a young orchard, and even though some fruit may be produced, the long term consequences of unfilled orchard space could be disastrous. Although remedial applications of counteracting hormones may alleviate the effects of some growth regulators when both are applied at the same time (Tromp 1982), it is not known whether or not such applications would work once one of the growth regulators had already taken effect. Much more investigation would be needed in this area before such chemicals could be used with complete confidence on very young, extensively planted orchards.

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